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## Temporal Compartmentalization of Viral Infection in Bacterial Cells

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2 **Main Manuscript for**

3 Temporal Compartmentalization of Viral Infection in Bacterial Cells

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21  
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25  
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27 bacteriophage; phage infection; virus infection; viral DNA replication; phage DNA replication; virus  
28 assembly; bacterial cell compartmentalization; Gram-positive bacterium; phage-host interaction; virus-  
29 host interaction

30  
31 **Author contributions**

32 A.L., L.J., C.B., B.F., M.L.-S., P.P., E.J., and P.T. conducted experiments. C.B. developed image  
33 analysis tools. All authors interpreted data. A.L., C.B., E.J., L.J., and B.F. prepared Figures. P.T., A.L.,  
34 R.C-L., and S.A. wrote the manuscript.

35

36 **This PDF file includes:**  
37 Main Text  
38 Figures 1 to 6  
39

#### 40 **Abstract**

41 Virus infection causes major rearrangements in the subcellular architecture of eukaryotes but its  
42 impact in prokaryotic cells was much less characterized. Here, we show that infection of the bacterium  
43 *Bacillus subtilis* by bacteriophage SPP1 leads to hijacking of host replication proteins to assemble  
44 hybrid viral-bacterial replisomes for SPP1 genome replication. Their biosynthetic activity doubles the  
45 cell total DNA content within 15 min. Replisomes operate at several independent locations within a  
46 single viral DNA focus positioned asymmetrically in the cell. This large nucleoprotein complex is a self-  
47 contained compartment whose boundaries are delimited neither by a membrane nor by a protein cage.  
48 Later during infection, SPP1 procapsids localize at the periphery of the viral DNA compartment for  
49 genome packaging. The resulting DNA-filled capsids do not remain associated to the DNA transactions  
50 compartment. They bind to phage tails to build infectious particles that are stored in warehouse  
51 compartments spatially independent from the viral DNA. Free SPP1 structural proteins are recruited to  
52 the dynamic phage-induced compartments following an order that recapitulates the viral particle  
53 assembly pathway. These findings show that bacteriophages restructure the crowded host cytoplasm to  
54 confine at different cellular locations the sequential processes that are essential for their multiplication.

#### 55 **Significance Statement**

56 Virus lytic infection disrupts host cell homeostasis and takes over cell space for virus multiplication.  
57 . Viral-induced reorganization of prokaryotic cells must meet the challenge to restructure the cytoplasm  
58 open space of a small-sized cell. We discovered that the bacterial siphovirus SPP1 builds two types of  
59 independent compartments in the host cytoplasm to confine viral DNA enzymatic reactions and to store  
60 viral particles. This spatial partition responds to the requirements for exponential replication of SPP1  
61 genomes and for assembly of hundreds of viral particles. Its similarities to remodelling of the cell  
62 nucleus by herpesviruses led to the hypothesis that ancestral strategies used by viruses to invade the  
63 cell space were conserved to infect hosts of different Domains of Life.

64

#### 65 **Main Text**

##### 66 **Introduction**

67 Organization of the cellular space is a common feature of living systems. Cell compartments  
68 delimited by membranes were observed in eukaryotic cells at the dawn of cell biology. More recently,  
69 membrane-less self-organizing compartments were identified, adding further spatial order to the cell.

70 Bacterial cells mainly rely on the latter type of strategy to structure their “open-space” cytoplasm  
71 because they rarely feature intracellular membranes (1), although some notable exceptions exist (2).  
72 One well-documented case of membrane-free compartmentalization is self-assembly of protein cages  
73 that confine biochemical reactions (3) or store specific cargo (4) in the bacterial cytoplasm. Considering  
74 their increasing variety, cell compartments are collectively defined here as a self-contained space that  
75 concentrates a set of macromolecular components inside the cell. Their boundaries can be established  
76 by membranes, a protein cage, or by the physicochemical properties of their components.

77 Lytic viral infection disrupts host cell homeostasis, re-allocating extensive resources for virus  
78 multiplication. In eukaryotic viruses this takeover correlates with the formation of virus replication  
79 compartments either in the host cytoplasm or in the nucleus (5). These viral factories can be  
80 membrane-associated structures as in case of several RNA virus families (6), membrane-less viral  
81 DNA replication compartments (5), or liquid compartments formed by phase separation (7). Re-  
82 structuration of bacterial cell architecture upon infection by bacterial viruses (phages or bacteriophages)  
83 is much less documented. DNA replication has been reported to occur at particular positions of the  
84 bacterial cytoplasm for four *Caudovirales*, a widely distributed order of double-stranded DNA (dsDNA)  
85 viruses also known as tailed bacteriophages. Phage  $\phi$ 29 uses the actin-like MreB cytoskeleton and the  
86 bacterial nucleoid to position protein-primed replication of its ~20 kbp genome at the periphery of the  
87 host cytoplasm (8, 9). Jumbo phages compartmentalize replication and transcription of their >200 kbp  
88 genome inside a viral proteinaceous nucleus-like cage that is positioned at mid-cell by a tubulin-related  
89 spindle (10–12). These are rare cases within the tailed phagesphere because of their particular mode  
90 of protein-primed replication ( $\phi$ 29) and of their genome size ( $\phi$ 29 and jumbo phages). The genomes of  
91 sequenced tailed phages have a median length of 51 kbp and those of the *Siphoviridae* family, which  
92 accounts for more than 55% of *Caudovirales*, are 48 kbp-long on median (13).  $\lambda$  and SPP1, which typify  
93 the siphoviruses, accumulate their replicated DNA in a defined focus localized asymmetrically in the cell  
94 during lytic infection (14, 15) but further investigation is critical to assess the impact of their infection on  
95 cell biology.

96 Here we investigate cell host takeover by the well-characterized bacteriophage SPP1 (16–20),  
97 which infects the Gram-positive model bacterium *Bacillus subtilis* (Fig. 1A). We discovered that phage  
98 infection leads to an extensive reorganization of the *B. subtilis* cytoplasmic space. SPP1 is shown to  
99 assemble two temporally and spatially independent types of compartments in the bacterial cytoplasm. A  
100 single compartment harbors viral genome replication and packaging while the others are storage  
101 regions of viral particles (or virions).

102

## 103 **Results**

104 **DNA biosynthetic challenge imposed by bacteriophage SPP1 infection to the host cell.** Genome  
105 replication and virion assembly are the key steps of virus multiplication (Fig. 1A) accounting for most of  
106 phage infection biosynthetic costs. To investigate their impact on the architecture of infected cells, we  
107 used a phage that carries an array of ~64 *lacO* operator sites inserted in its genome  
108 (SPP1~~*IX110lacO64*~~). By infecting *B. subtilis* cells producing the cognate LacI binding protein fused to  
109 a fluorescent protein (FP), phage DNA can be visualized during infection (15). SPP1~~*IX110lacO64*~~  
110 produces ~2 to 3-fold less progeny virions than SPP1 wild-type but impacts neither the phage temporal  
111 infection program nor the timing of phage lysis (*SI Appendix*, Fig. S1A) (15, 16). SPP1~~*IX110lacO64*~~,  
112 abbreviated SPP1~~*lacO64*~~ from hereafter, and the *B. subtilis* strain producing LacI-mCherry are the  
113 parental viral and bacterial strains, respectively, used throughout the present study unless stated  
114 otherwise.

115 The DNA mass in SPP1~~*lacO64*~~-infected cells rose sharply after 15 min post-infection (p.i.)  
116 (*SI Appendix* Fig. S1B). At 30 min p.i., when lysis initiates in rich medium cultures grown at 37°C, total  
117 DNA increased ~2-fold relative to non-infected cells. The mean cell surface did not vary significantly  
118 during infection ( $2.71 \pm 0.78 \mu\text{m}^2$  (n=2464) in infected cells vs  $2.85 \pm 0.79 \mu\text{m}^2$  (n=1160) in non-infected  
119 cells), indicating that the DNA/cytoplasmic space ratio was doubled. We next quantified bacterial and  
120 viral DNA during infection by qPCR. Viral genome replication was detectable at 10 min p.i. and  
121 increased exponentially until 30 min p.i. (Fig. 1B). At this stage of infection each bacterium contained  
122 an average of 318 copies of the phage genome (Fig. 1B, empty circles) accounting for 13.4 Mbp of viral  
123 DNA, which exceeds the ~11 Mbp of bacterial DNA present in the cell (equivalent to 2.7 copies of the  
124 4.06 Mbp *B. subtilis* YB886 genome (*SI Appendix* Fig. S1 C and D) (21)).

125 Genome encapsidation quantified by qPCR of DNase-protected DNA was detected at 20 min p.i.  
126 (Fig. 1B, filled circles) due to its temporal delay relative to DNA replication (16). Only a fraction of the  
127 SPP1 DNA synthesized (reaching ~60% at 30 min p.i.) was encapsidated inside viral particles (Fig. 1B,  
128 filled circles and Fig. 1C). The majority of those particles (~75% at 30 min p.i.) were infectious as  
129 quantified in a phage titration plaque assay (Fig. 1B, squares). The non-infectious viral particles were  
130 likely DNA-filled capsids that did not yet attach tails in their assembly pathway (22) and possibly some  
131 defective tailed phage particles. Interestingly, SPP1 DNA synthesis was reduced in infections with a  
132 phage defective in the DNA packaging enzyme gp2 (SPP1~~*IX110lacO64sus19*~~ (Table S1) (23),  
133 hereafter abbreviated as SPP1~~*lacO64gp2*~~) that does not encapsidate DNA (Fig. 1D), indicating a  
134 regulatory feedback between genome encapsidation and DNA replication.

135

136 ***B. subtilis* replisome proteins are recruited into hybrid phage-host replisomes at the viral DNA**  
137 **compartment.** Mono-infected *B. subtilis* cells display a single phage DNA focus (*SI Appendix* Fig. S2 A

138 and *B*), hereinafter referred to as the viral DNA compartment, which increases in size during infection  
139 (*SI Appendix Fig. S2B*) (15). Since SPP1 requires several host proteins for replication of its genome  
140 (Table S2) (24-26), we investigated the sub-cellular localization of both SPP1 DNA and FP fusions to *B.*  
141 *subtilis* replisome components (Fig. 2*A* and *SI Appendix Fig. S2C*). In non-infected bacteria the DNA  
142 polymerase PolC, the clamp loader subunit DnaX, the sliding clamp DnaN, and the single-stranded  
143 DNA binding protein SsbA localized into small foci, presumably the *B. subtilis* replisomes assembled at  
144 replication forks (arrowheads in Fig. 2*A* and *SI Appendix Fig. S2C*) (27). Foci of the primosome protein  
145 DnaB and of the DNA polymerase DnaE were also detected in some cells, while the primase DnaG  
146 displayed a diffuse distribution (*SI Appendix Fig. S2C*, left) (27, 28). Interestingly, in infected bacteria all  
147 seven host proteins co-localized with SPP1 DNA, irrespectively of being essential (PolC, DnaE, DnaX,  
148 DnaN and DnaG) or dispensable (SsbA and DnaB) for SPP1 multiplication (Fig. 2 and *SI Appendix*  
149 *Fig. S2C* and Table S2). The co-localization of DnaE and DnaB fluorescent protein fusions with phage  
150 DNA was more prominent early p.i. while the other host replisome components associated persistently  
151 to phage DNA during infection. Small foci of SsbA and more rarely of DnaX were also detected outside  
152 the viral DNA compartment in some infected cells (arrows in Fig. 2*A* and *SI Appendix Fig. S2C*)  
153 possibly associated to host DNA. The *B. subtilis* replicative helicase DnaC showed a homogeneous  
154 distribution both in non-infected and in infected bacteria (Fig. 2*B* and *SI Appendix Fig. S2C*).

155 SPP1 infection led to a loss of the regular distribution of bacterial DNA replication origins in the cell  
156 (*SI Appendix Fig. S1E*) but replication of the *B. subtilis* genome continued at least early during phage  
157 infection (*SI Appendix Fig. S1F*). Nevertheless, the prominent spatial concentration of bacterial DNA  
158 replication effectors in the SPP1 DNA compartment correlated with a strong viral genome replication  
159 activity (Fig. 1*B* and *SI Appendix Fig. S1G*) that outperforms DNA replication in the bacterial nucleoid  
160 (Fig. 1*B*). We concluded that the DNA replication machinery of *B. subtilis* is biochemically and spatially  
161 re-programmed for SPP1 genome replication.

162 We next investigated how the host replisome is recruited to the phage DNA compartment in three-  
163 color imaging experiments in which SPP1 DNA, *B. subtilis* replication proteins, and SPP1 replication  
164 proteins were simultaneously labelled. SPP1 DNA replication is initiated by gp38 binding and melting of  
165 the phage origins of replication (29). Then, gp39 loads the helicase gp40 to the open origins (26, 30).  
166 Gp40 is known to interact with the host clamp loader subunits DnaX (31) and with the primase DnaG  
167 (32, 33). The finding that a gp40-mCitrine fusion produced in non-infected *B. subtilis* displayed a  
168 replisome-like localization and co-localized with mCFP-DnaX (Fig. 3*A*) is consistent with those  
169 previously identified interactions. Thus, the SPP1 helicase is recruited to the *B. subtilis* replisome in the  
170 absence of other phage proteins. In infected cells, both gp40 and DnaX relocalized into two or more  
171 foci inside the SPP1 DNA compartment (Fig. 3*B*), as also observed for other host replisome

172 components (Fig. 2A and *SI Appendix* Fig. S2C). These results indicate that viral DNA replication  
173 factories composed of hybrid phage-host replisomes assemble at distinct positions within the viral DNA  
174 compartment.

175 Upon entry into the host cell, the SPP1 genome is circularized and undergoes several cycles of  
176 circle-to-circle (theta) replication, leading to an exponential increase of phage DNA template molecules,  
177 and then switches to a linear sigma replication mode to generate genome concatemers (Fig. 3E). The  
178 gp40 helicase is essential for SPP1 DNA synthesis in both modes (25, 26) providing a hallmark for  
179 localization of SPP1 replication throughout infection (Fig. 3F). The SPP1 recombinase gp35 is essential  
180 for the theta-to-sigma replication mode switch and the 5'-3' exonuclease gp34.1 is the other major  
181 phage effector of this step (17, 34). We were unable to determine the subcellular localization of gp35  
182 due to aggregation of different FP fusions to gp35, but we obtained a functional gp34.1-mCitrine fusion.  
183 Gp34.1-mCitrine formed a small focus that increased in size and co-localized with phage DNA in  
184 infections with a SPP1 mutant defective for production of gp34.1 (Fig. 3G). Thus, the complete  
185 sequential program of SPP1 replication is compartmentalized in the phage DNA compartment.

186

187 **Viral DNA occupies a self-contained dynamic compartment.** Presence of a single viral DNA  
188 compartment in mono-infected bacteria indicates that replication of the SPP1 DNA molecule, which  
189 entered the cell at the beginning of infection (Fig. 1A), occurs at a specific location in the cytoplasm  
190 where descendent viral genomes accumulate throughout infection (*SI Appendix* Fig. S2 A and B). We  
191 then analyzed quantitatively this compartment position and shape in hundreds of mono-infected cells.  
192 The compartment grew as infection proceeded (Fig. 4A) and had a center of mass at about 1.2  $\mu\text{m}$  from  
193 the cell proximal pole regardless its size (Fig. 4B). The cell diameter of *B. subtilis* ( $\sim 1 \mu\text{m}$ ) rapidly  
194 constrained radial growth of the compartment leading to an elongated shape. Its area increased by  
195 longitudinal growth as DNA synthesis ensued (Fig. 4 C-E). Phage DNA accessible for LacI-mCherry  
196 labeling occupied, on average, 25% of the cell area late in infection (Fig. 4A, blue). This accounted only  
197 for  $\sim 40\%$  of the total SPP1 DNA synthesized because the remaining DNA shielded inside viral particles  
198 (Fig. 1B,C) was stripped-off of LacI-mCherry during its packaging into phage procapsids through a 30  
199 Å-wide portal channel. To visualize the total SPP1 DNA synthesized we infected cells with  
200 SPP1/*acO64gp2'* that is defective in DNA packaging. In absence of genome encapsidation, the viral  
201 DNA compartment grew into a significantly more elongated shape (Fig. 4 C-E) revealing that SPP1  
202 DNA can occupy more than 35% of the cell area (Fig. 4A, magenta).

203 In order to visualize this impact of SPP1 infection on DNA spatial distribution within the cell  
204 ultrastructure we carried out immunoelectron microscopy (immunoEM) of thin sections from *B. subtilis*  
205 YB886, an isogenic strain without fluorescent markers. DNA was localized using an anti-DNA

206 monoclonal antibody decorated with 5 nm colloidal gold (small dots in Fig. 5 A,B,E and F) or DNase  
207 treatment (Fig. 5 C and D) (35). In non-infected cells two separated, symmetrically positioned, bacterial  
208 nucleoids were readily observable (Fig. 5 A,C and E), consistent with DAPI fluorescence staining (Fig.  
209 5G). SPP1/*lacO64gp2<sup>-</sup>* infection led to a major reorganization of the cytoplasmic space where DNA  
210 occupied a much larger region (Fig. 5D). Loss of the bacterial nucleoids typical position and  
211 organization in the cell (Fig. 5 B,D,F and H) correlated with re-positioning of *B. subtilis* chromosome  
212 origins away from the viral DNA compartment (*SI Appendix* Fig. S1E).

213 Membrane staining shows that no membrane separates the SPP1 DNA compartment from the  
214 cytoplasm (*SI Appendix* Fig. S2B) (15). The unpackaged ~125 genome copies (5.3 Mbp) of  
215 SPP1/*lacO64* DNA late p.i. (Fig. 1B) occupy no more than ~25% of the cytoplasm open space area (Fig.  
216 4A). To investigate if integrity of this viral DNA compartment requires active DNA replication and is  
217 energy-dependent we arrested DNA polymerase III activity with 6 (*p*-Hydroxyphenylazo)-Uracil (HPUra)  
218 (15, 36) and collapsed the proton motive force with 2,4-dinitrophenol (DNP) (37), respectively. Both  
219 HPUra and DNP prevented formation of the viral DNA compartment at the beginning of infection. When  
220 the drugs were added after appearance of the SPP1 DNA focus, they did not disrupt its morphological  
221 integrity (Fig. 3 C and D; SPP1 DNA panels). In HPUra-treated cells, proteins of the hybrid viral-  
222 bacterial replisome (visualized by gp40 and DnaX) lost, however, their typical pattern of discrete small  
223 foci within the DNA compartment and re-localized all over the SPP1 DNA (Fig. 3C). We concluded that  
224 maintenance of SPP1 replication factories, but not of the viral DNA compartment, required active DNA  
225 synthesis. In DNP-treated cells, DnaX lost most of its association with the compartment and displayed a  
226 patchy pattern in the cytoplasm while gp40 maintained its co-localization with SPP1 DNA (Fig. 3D).  
227 Thus, energy depletion leads to hybrid replisomes disassembly and dissociation of DnaX, but not of  
228 gp40, from the viral DNA compartment.

229

230 **A sub-population of SPP1 procapsids localizes at the edges of the viral DNA compartment.** The  
231 goal of viral genome replication is to provide a nucleic acid substrate suitable for viral particles  
232 construction. In tailed bacteriophages, as in numerous viruses, replicated DNA is packaged into  
233 procapsids that are virion precursors without DNA (Fig. 5K), prompting investigation of their position  
234 relative to DNA in the cell. Procapsids accumulate in the cytoplasm of bacteria infected with SPP1  
235 mutants defective for genome packaging like SPP1/*lacO64gp2<sup>-</sup>* (18). Reliable imaging of individual  
236 phage procapsids and virions by EM of infected bacteria was hampered by sample fixation, in spite of  
237 extensive optimization work. However, immuno-labeling of thin sections with anti-(pro)capsid antibodies  
238 showed that those structures (thick dots in Fig. 5 B,D and F) accumulate at the periphery of DNA (thin  
239 dots in Fig. 5 B and F) in *B. subtilis* YB886 cells infected with SPP1/*lacO64gp2<sup>-</sup>*. Procapsid labeling was

240 reproducibly enhanced by digestion of DNA (Fig. 5D) and of trypsin-sensitive non-assembled proteins  
241 (Fig. 5F) in the thin sections.

242 We then imaged specifically SPP1 DNA with LacI-mCherry and the procapsid scaffolding protein  
243 gp11 fused to mCitrine in live cells. Gp11 is a rod-like internal chaperone that scaffolds multimerization  
244 of the major capsid protein gp13 to build functional procapsids (38). Gp11 then exits the procapsid  
245 through holes of  $\sim 3 \times 2.2$  nm in the gp13 protein lattice to leave space for DNA packing (Fig. 5K) (39).  
246 Infection of cells producing mCitrine-gp11 with phages coding for gp11 but defective in DNA packaging  
247 (e.g. SPP1/*lacO64gp2<sup>-</sup>* in Fig. 5K) led to accumulation of procapsids in the bacterium. Their  
248 sedimentation behavior and shape were similar to SPP1 wild-type procapsids although they carry the  
249 two gp11 forms (*SI Appendix* Fig. S3 A and B), showing that mCitrine-gp11 is incorporated into  
250 correctly-shaped procapsids during assembly. Unexpectedly, CsCl-purified phage particles produced  
251 during infection with SPP1/*lacO64* contained mCitrine but not the full-length mCitrine-gp11 fusion (*SI*  
252 *Appendix* Fig. S3C). We posited that the mCitrine  $\beta$ -barrel dimensions ( $\sim 3 \times 4$  nm size in case of  
253 mCitrine (40)) precluded exit of mCitrine-gp11 through the procapsid holes and interpreted that the  
254 fusion protein was proteolyzed when its gp11 moiety left the procapsid prior to DNA packaging, leaving  
255 mCitrine stuck inside. Collectively, the data show that mCitrine-gp11 is incorporated into procapsids  
256 and that mCitrine remains inside functional structures during their subsequent maturation steps until the  
257 end of viral particle assembly.

258 mCitrine-gp11 distributed homogeneously in the cytoplasm of either non-infected cells (Fig. 5I) or  
259 cells infected with SPP1/*lacO64gp13<sup>-</sup>* (*SI Appendix* Fig. S3D), a mutant defective in the major capsid  
260 protein gp13 that does not build procapsids (Fig. 5K). When procapsids assembled during infection,  
261 mCitrine-gp11 formed foci in the cell consistent with its incorporation in viral structures. In absence of  
262 DNA packaging these foci accumulated at the phage DNA compartment periphery (Fig. 5J and  
263 *SI Appendix* Fig. S3 E and F) as found in immunoEM experiments (Fig. 5 B,D and F). To follow the  
264 localization of procapsids during infection without the space constraints imposed by the cells small size,  
265 we performed time-lapse observation of mCitrine-gp11 in filamentous *B. subtilis* bacteria infected by  
266 SPP1/*lacO64gp2<sup>-</sup>*. Septation was inhibited by adding the FtsZ-inhibitor PC190723 to exponentially  
267 growing cells prior to infection, leading to the formation of long, aseptated filaments (41). The SPP1  
268 DNA compartment became several micrometer-long in the filamentous cells before procapsids  
269 progressively accumulated at the compartment edges as well as at SPP1 DNA-free regions near the  
270 cell poles (Fig. 5L). Procapsids lacking the portal protein gp6 (42) showed a similar distribution (Fig.  
271 5M). Interestingly, when the bacterium lysed procapsids maintained their cluster organization in the  
272 agarose pad gel used to immobilize the cells while the SPP1 DNA compartment lost its organization  
273 (Fig. 5L, bottom panel arrow).

274 The localization pattern of mCitrine-gp11 had some distinctive features in cells infected by  
275 SPP1/*lacO64*, in which the assembly pathway of viral particles is complete. Fluorescent foci that flank  
276 the SPP1 DNA compartment are significantly better individualized and separated from the DNA  
277 compartment (*SI Appendix Fig. S3G*) than those observed when only procapsids accumulate in the  
278 infected cell (*SI Appendix Fig. S3 E and F*). Since the fluorescent protein reports the localization of both  
279 procapsids and DNA-filled capsids (see above) its assignment to the distribution of specific viral  
280 structures in the cell was not made.

281  
282 **SPP1 DNA-filled particles accumulate in spatially independent compartments.** In order to image  
283 SPP1 capsids specifically at the post-DNA packaging state, we fused mCitrine to the non-essential  
284 capsid accessory protein gp12 (Fig. 6A). The binding sites of gp12 in the gp13 capsid lattice are  
285 generated during DNA encapsidation (39, 43). Consistently, when *B. subtilis* cells expressing gp12-  
286 mCitrine were infected with a DNA packaging defective mutant, gp12 displayed a diffuse localization in  
287 the cytoplasm (Fig. 6B). During productive infections, gp12-mCitrine bound to DNA-filled viral particles  
288 and localized to foci flanking the phage DNA compartment both in the absence (Fig. 6C) and in the  
289 presence (Fig. 6D) of phage-encoded gp12. This spatial distribution correlated with the presence of two  
290 electron-dense regions near the poles of infected cells in immunoEM thin sections that were labelled  
291 with anti-DNA and anti-capsid antibodies (Fig. 6 E and G). Infections for EM and DAPI DNA-labeling  
292 experiments (Fig. 6 E-H) were carried out in an isogenic strain producing no fluorescent proteins to  
293 exclude the possibility that reporter fusion proteins affect the formation or properties of foci containing  
294 DNA-filled capsids. Therefore, formation of such foci and their stronger prominence in absence of gp12  
295 are inherent features of SPP1 viral particles at the post-DNA packaging state (compare Fig. 5 A,B,G  
296 and H to Fig. 6 E-H). In thin sections, those particles were almost certainly sectioned during preparation  
297 rendering their DNA accessible to labeling (Fig. 6 E and G). The less compact DNA present at more  
298 central regions of the cell corresponds presumably to both SPP1 and host free DNA (Fig. 6 E and G).

299 We then made triple-labeling experiments to follow the spatial distribution of capsids in the cell  
300 during their maturation from the DNA packaging step to the formation of mature virions after addition of  
301 the tail (Fig. 6A). Capsids were labeled with gp12-mCFP and tails with the major protein gp17.1 (44)  
302 fused to mCitrine in filamented cells infected with SPP1 mutants impairing different assembly steps of  
303 the viral particle (Fig. 6A and *SI Appendix Fig. S4*). When DNA packaging was arrested, gp12-mCFP  
304 remained homogenous in the bacterial cytoplasm while foci of gp17.1-mCitrine flanked the SPP1 DNA  
305 compartment (*SI Appendix Fig. S4A*). These foci, which were absent in the infection with a SPP1  
306 mutant defective in tail formation (*SI Appendix Fig. S4B*), reveal regions of tail structures clustering.  
307 During infection with SPP1/*lacO64gp16* the post-DNA packaging step that leads to formation of the

308 capsid connector interface necessary for tail binding is disrupted (45). In such situation, both capsids  
309 (gp12-mCFP label) and tails (gp17.1-mCitrine label) formed foci outside the region occupied by the  
310 SPP1 DNA compartment. Those foci were mostly spatially independent, as can be best appraised on  
311 the rightmost overlay of gp12-mCFP (blue) and gp17.1-mCitrine (yellow) signals in *SI Appendix*  
312 Fig. S4C. We conclude that capsids and tails accumulate predominantly in different discrete locations  
313 within the cell when their interaction is impaired. In contrast, such segregation is not detectably  
314 observed when assembly of tailed phage particles occurs in cells infected with SPP1*lacO64* (Fig. 6I).  
315 Time-lapse experiments showed that DNA-filled capsids and tails co-localize as soon as they become  
316 detectable, revealing a rapid capsid-tail association in wild-type infection conditions (*SI Appendix* Fig.  
317 S4D). Therefore, the spatial distribution of SPP1 capsids and tails recapitulates precisely the post-DNA  
318 packaging sequential assembly steps (Fig. 6 A and I and *SI Appendix* Fig. S4). We defined the  
319 compartments where mature virions are stored as warehouses.

320 At early infection times, the SPP1 DNA overlapped with the most proximal warehouse identified by  
321 the gp12 focus in ~50% of the cells (Fig. 6J). Late in infection, most gp12-mCitrine had re-localized to  
322 positions distant from the SPP1 DNA (Fig. 6 C,D and J). Automated segmentation of fluorescent gp12-  
323 mCitrine foci in mono-infected cells confirmed that there are multiple foci early in infection, which likely  
324 merge afterwards to form two or three warehouses (*SI Appendix* Fig. S5A). In cells containing two  
325 warehouses, these were generally found on either side of the viral DNA compartment  
326 (*SI Appendix* Fig. S5B) at different relative distances (*SI Appendix* Fig. S5 C and D). Their fluorescence  
327 intensity revealed a roughly similar distribution of DNA-filled particles between the two warehouses  
328 (*SI Appendix* Fig. S5E). We next analyzed the gp12-mCitrine focus proximal to the viral DNA  
329 compartment to follow the evolution of warehouses during infection. The surface of the warehouses  
330 increased moderately during infection (*SI Appendix* Fig. S5F) and they remained quasi-spherical  
331 (*SI Appendix* Fig. S5G). However, their fluorescence intensity raised significantly (*SI Appendix* Fig.  
332 S5H) indicating that DNA-filled particles adopt a more compact organization in the warehouse with  
333 time. Gp12-mCitrine foci formed in the absence of phage-encoded gp12 were more compact and  
334 brighter than the ones built in the presence of phage-encoded gp12 (*SI Appendix* Fig. S5 F-H).

335 We next visualized the complete spatio-temporal program of viral DNA compartment and virion  
336 warehouses formation during infection of gp12-mCitrine-producing cells with SPP1*lacO64gp12* in real  
337 time using a microfluidics device (Fig. 6K and Movie S1). Quasi-circular phage DNA compartments  
338 formed first and then elongated throughout infection until cell lysis. Flanking, spatially independent,  
339 warehouses of DNA-filled particles appeared later. They grew initially to a defined circular shape and  
340 then became more intense without major change in their size. Interestingly, bacteria continued to  
341 elongate and to divide during infection (white arrow in Fig. 6K), although their growth in liquid medium

342 was slightly reduced relative to non-infected bacteria (*SI Appendix* Fig. S1B). In some cells, the division  
343 septum sectioned the viral DNA compartment partitioning phage genome copies between daughter  
344 cells in which SPP1 DNA continued to replicate (Fig. 6L).

345

## 346 **Discussion**

347 We have discovered that bacteriophage SPP1 infection generates compartments in the crowded  
348 bacterial cytoplasm that have neither membrane nor protein cage boundaries. The spatio-temporal  
349 independent compartments confine viral DNA transactions and storage of viral particles. This spatial  
350 partition responds to the requirements for accommodating exponential replication of the viral genome  
351 and assembly of hundreds of infectious viral particles, the two central processes of the viral lytic cycle.  
352 The resulting major quantitative perturbation is the amount of viral DNA synthesized, which rises >2-  
353 fold the total cell DNA mass (Fig. 1B; Dataset S1). Concomitantly, bacterial nucleoids lose their regular  
354 position and condensed organization in the cell (Fig. 5 A-H and *SI Appendix* Fig. S1E). These changes  
355 in the *B. subtilis* chromosomes organization can be attributed to arrest of its replication (46) by hijacking  
356 bacterial replisomes to replicate SPP1 DNA (Fig. 2 and *SI Appendix* Fig. S2C), although some bacterial  
357 DNA synthesis still proceeds during infection (*SI Appendix* Fig. S1F). The area occupied by DNA in  
358 infected bacteria increases without a detectable change on average cell size in spite of the mechanism  
359 that maintains a constant nucleocytoplasmic ratio across bacteria, including *B. subtilis* (47). Hence, viral  
360 DNA can occupy more than 35% of the cell area (Figs. 4A and 5D). Such spatial stress is relieved by  
361 DNA packaging, which strips-off proteins bound to the viral DNA to tightly pack it in capsids at a  
362 concentration of ~415 mg/mL, liberating space for higher yield DNA synthesis (Fig. 1D).

363 The single SPP1 DNA molecule entering the cytoplasm of mono-infected bacteria seeds the viral-  
364 induced DNA compartment found at a unique asymmetric position in the cell. This finding shows that  
365 the 44 kbp circular molecules generated by initial theta replication of the SPP1 genome (Fig. 3E) do not  
366 segregate to establish independent replication compartments. They rather stay confined together at a  
367 defined position of the cell. Similarly, eukaryotic herpesviruses, which are phylogenetically related to  
368 tailed phages (48) assemble discrete DNA replication compartments in the cell nucleus, each  
369 originating from a single incoming genome (49). Biogenesis of the viral DNA compartment can be  
370 partially explained by accumulation of replicated SPP1 DNA at a defined position of the cytoplasm.  
371 Polymer dynamics simulations predict that long DNA molecules fold to a compact globular shape in a  
372 crowded confined space like the cellular environment (1). Further compaction is likely achieved by  
373 protein-DNA interactions (e.g. with nucleoid-associated proteins), while binding of other proteins may  
374 antagonize it to ensure accessibility of genome sequences to DNA transactions like DNA replication,  
375 recombination, transcription, or encapsidation into viral procapsids (1, 50–52). These combined effects

376 can explain formation of a self-contained compartment with neither membrane nor protein cage  
377 boundaries. Its constrained metastable state is nearly instantaneously lost upon infection-induced cell  
378 lysis and death (e.g. 82 min p.i. in Fig. 6K and Movie S1), probably due to reduction of cytoplasmic  
379 crowding following leakage of the cell content. We also showed that the division septum can cut  
380 through the SPP1 DNA compartment during cell division, delivering viral genomes to the two daughter  
381 cells (Fig. 6L). This is advantageous for the phage, rendering superfluous an occlusion mechanism  
382 similar to the one employed by bacteria to prevent sectioning of their chromosome (53).

383 We found that SPP1-host hybrid replisome components co-localize at multiple discrete foci inside  
384 the viral DNA compartment. In contrast to the compartment integrity, stable maintenance of those  
385 punctuate hybrid replisome foci is DNA replication and energy-dependent (Fig. 3 B-D). We posit that  
386 they correspond to parallelized factories synthesizing DNA from several independent templates (Fig.  
387 3E), which are necessary to sustain the viral genome replication rate during infection. Considering that  
388 SPP1*lacO64* synthesizes 278 copies of its 42.3 kbp genome within 15 min (15-30 min p.i. in Fig. 1B)  
389 this corresponds to an average synthesis rate of 13.1 kbp/s. There are thus at least 13 fully operative  
390 DNA replication machines synthesizing at a rate of 1000 bp/s (54) distributed at different locations  
391 within the DNA compartment ultrastructure. The localization of procapsids at the periphery of the SPP1  
392 DNA compartment (Fig. 5) identifies the most likely position of replicated DNA packaging reactions into  
393 procapsids. A related spatial organization was found in the nucleus of cells infected by the human  
394 herpes simplex virus 1 (HSV-1) whose procapsids cluster at positions adjacent to the virus DNA  
395 replication centers (55). Presence of DNA replication and of DNA packaging molecular machineries in  
396 the same compartment provides a cytological basis for their cross-talk as reported for phages T4 (56)  
397 and SPP1 (57), as well as for the regulatory feed-back we observed between the two processes (Fig.  
398 1D). The two machineries must also work together in bacteriophages T3, T7 and T5, which require re-  
399 synthesis of DNA concomitantly with genome packaging to generate terminal repetitions of identical  
400 sequence in the DNA molecules encapsidated (58). Collectively, our data rationalize previous  
401 observations in several viral systems, defining the viral-induced DNA compartment as the central  
402 industrial hub to sequester the proteins required for DNA transactions and to couple spatially their  
403 reactions in the infected cell. It also reveals a strategy to confine viral DNA processes different from the  
404 protein cage used by jumbo phages to compartmentalize their DNA at mid-cell (10,11).

405 SPP1 capsids do not remain associated with the viral DNA compartment after DNA packaging, a  
406 morphogenetic step that is characterized by transition from the procapsid to the DNA-filled capsid state.  
407 This transition creates the binding sites for the non-essential auxiliary protein gp12 at the capsid  
408 surface (39, 43) and the interface for tails binding to the capsid connector. The latter reaction leads to  
409 formation of infectious virions (Fig. 6A). Virions accumulate in a different type of compartment, the

410 warehouse (Fig. 6 and *SI Appendix* Figs. S4 and S5), irrespectively of gp12 binding to capsids.  
411 Particles lacking gp12 form warehouses significantly more compact than those with gp12 do (Fig. 6 C-  
412 H). This finding shows that the properties of the phage icosahedral capsid surface play a main role on  
413 warehouse organization and possibly also on its biogenesis. We hypothesize that it is the smooth (59),  
414 albeit more electronegative surface of capsids lacking gp12 (43), that facilitates their tighter association  
415 in warehouses when compared to capsids decorated with arrays of flexible, symmetrically arranged,  
416 gp12 trimers (39, 59). Orderly packing of a large number of phage particles through their capsid  
417 icosahedral lattices is certainly disturbed by the presence of tail structures. Such constrain makes it  
418 unlikely to reach a crystal-like arrangement of viral particles as found for HSV-1 and polyomavirus  
419 capsids in the nucleus of infected eukaryotic cells (60, 61). The formation of phage warehouses fulfills,  
420 nevertheless, their putative function to minimize the storage space of viral particles in the cell.

421 The SPP1 DNA is in direct contact with the cytoplasm rendering it vulnerable to cellular defenses  
422 like CRISPR-Cas (62), in contrast to the nucleus-like protein cage used by jumbo-phages to protect  
423 their DNA (12, 63). Although this is a limitation for SPP1 exploration of hosts landscape, such cost is  
424 plausibly compensated by the competitive advantage of its much higher burst size and shorter infection  
425 period when compared to jumbo phages multiplication parameters (64). A different way to exploit the  
426 cytoplasm open space is typified by bacteriophage phage  $\phi$ 29 that targets the MreB bacterial  
427 cytoskeleton to structure its DNA replication at the periphery of the cell interior (8, 9). This strategy  
428 allows for production of a high burst of infectious virions but might be limited to the few viruses that  
429 replicate their DNA by a protein-primed replication mechanism. The SPP1 compartments properties  
430 offer a cost and time-effective way to exchange protein effectors between the cellular pool and viral  
431 compartments to fulfill the needs for phage multiplication. Proteins enrollment follows a defined order.  
432 This principle is well illustrated by assembly of SPP1 viral particles. Their building blocks are recruited  
433 from homogeneous cytoplasmic pools to a defined sub-cellular localization, following an order that  
434 recapitulates the program of viral particle sequential assembly. That is shown in our study for the  
435 scaffolding protein gp11 at the procapsid assembly stage (*SI Appendix* Fig. S3 D-G), the capsid  
436 accessory protein gp12 at the post-DNA packaging state (Fig. 6 B-D), and the tail fixation to the capsid  
437 connector interface (*SI Appendix* Fig. S4).

438 The ways how SPP1 restructures the infected cell are most probably used by a large number of  
439 phages. SPP1 partition of cytoplasmic space has also a number of striking similarities with remodeling  
440 of the eukaryotic cell nucleus during infection by herpesviruses (see above) (49, 55 and references  
441 therein). Those changes of cell architecture concur with common molecular mechanisms of  
442 recombination-dependent DNA replication (17) and packaging into viral procapsids (65). We propose

443 that SPP1 typifies ancestral strategies used by viruses to organize the cellular space that were  
444 conserved to infect hosts of different Domains of Life.

445

## 446 **Materials and Methods**

447 A complete list of bacterial strains, phages, plasmids, antibodies and primers used in this work is  
448 presented in *SI Appendix, Table S3*.

449 A detailed description of all the methods is provided in *SI Appendix, Materials and Methods* that  
450 includes bacterial, phages and plasmids construction; phages amplification and infection studies;  
451 purification and characterization of phage particles; qPCR, fluorescence microscopy, microfluidics, and  
452 quantitative imaging of infected bacteria; electron microscopy, and statistical analyses. The MatLab  
453 scripts used in this work and a tutorial were uploaded to a repository in GitHub for open access:  
454 [https://github.com/CyrilleBillaudeau/TemporalCompartmentalization\\_ofViral\\_Infection\\_inBactCells](https://github.com/CyrilleBillaudeau/TemporalCompartmentalization_ofViral_Infection_inBactCells).

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477

478 **Competing interests**

479 The authors declare that they have no competing interests.

480

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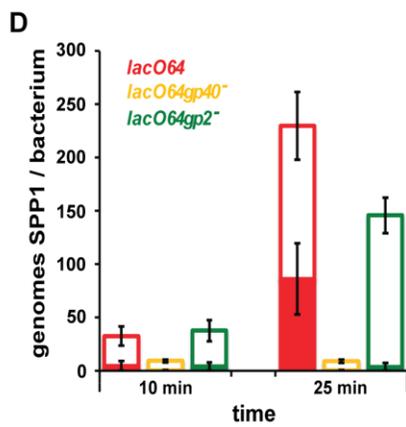
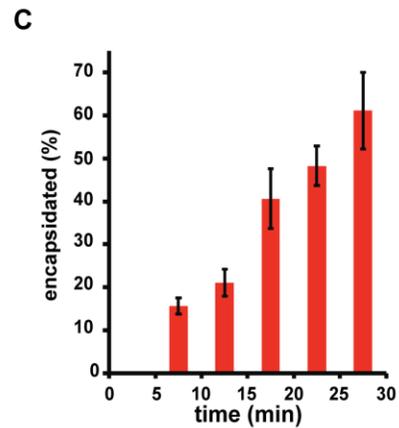
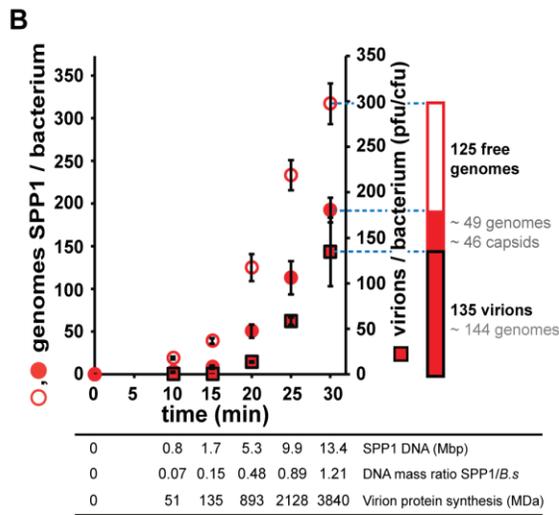
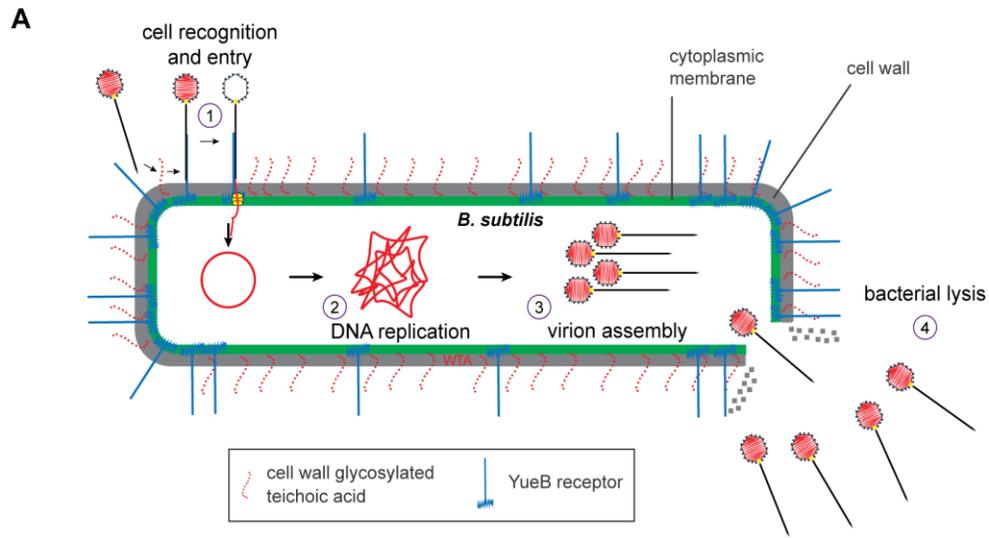
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1 **Figures**

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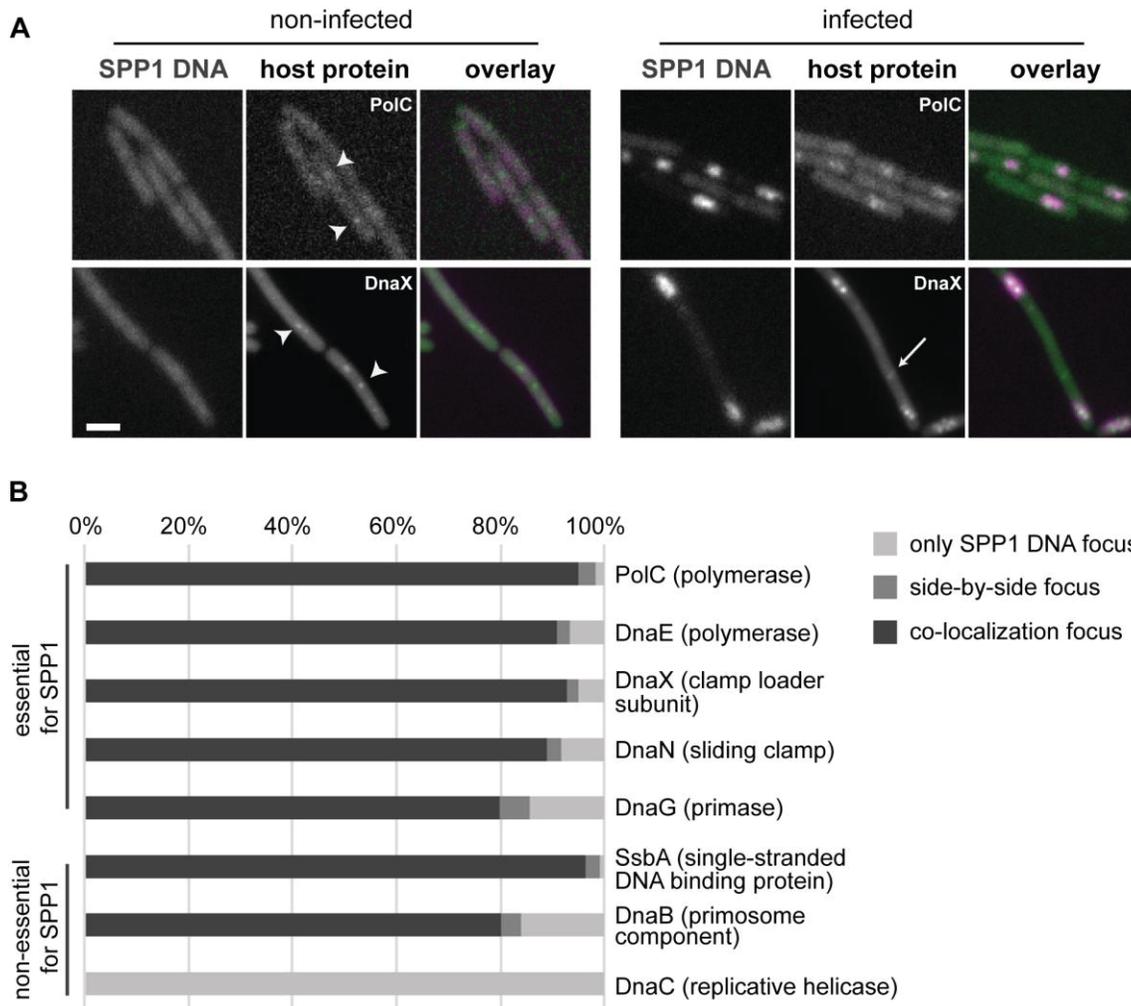
5 **Fig. 1. SPP1 DNA replication and encapsidation.**

6 (A) Schematics of the main steps on tailed bacteriophage infection exemplified by SPP1.  
7 (B) Quantification of SPP1 DNA synthesis (empty circles) and encapsidation (filled circles) determined  
8 by qPCR of total and DNase-protected DNA, respectively, in *B. subtilis* GSY10004 infected with  
9 SPP1/*lacO64*. The amount of SPP1 gene 6 DNA was divided by the amount of *B. subtilis gyrA* reporter  
10 DNA considering that there are 2.7 nucleoids on average *per cell* (*SI Appendix* Fig. S1 C and D) to  
11 calculate the number of SPP1 genomes *per bacterium*. The total number of infectious particles (filled  
12 squares; ordinate on the right) was quantified by phage titration of bacteria lysed with 10% chloroform.  
13 Note that the DNA molecule encapsidated in SPP1/*lacO64* particles (~45.1 kbp) is 1.07-fold longer than  
14 the phage genome size (42.3 kbp) (15, 66), a factor that was used to convert phage particle counts into  
15 genome equivalents and reciprocally. A snapshot of phage DNA and particle yields at 30 min p.i., when  
16 experiments were stopped because cell lysis initiated, is displayed on the right. Values in bold were  
17 obtained experimentally and those in grey were calculated based on the figure data. Note that the  
18 calculation of ~46 capsids corresponds to particles that protect viral DNA but are non-infectious (i.e.  
19 tailless DNA-filled capsids and eventually defective tailed phage particles). The data are the average from  
20 three independent experiments. The biosynthetic effort accounting for phage DNA and virion structural  
21 proteins synthesis is depicted underneath the infection time points (Dataset S1). (C) Percentage of  
22 encapsidated DNA calculated from the data in B. (D) Phage DNA synthesis at 10 and 25 min p.i. in  
23 *B. subtilis* GSY10004 infected with SPP1/*lacO64* (15) (red), SPP1/*lacO64gp40<sup>-</sup>* (defective in initiation of  
24 DNA synthesis) (yellow), and SPP1/*lacO64gp2<sup>-</sup>* (defective in DNA packaging) (green). Total DNA (bars  
25 total height) and encapsidated, DNase-protected DNA (overlapping full bars) were quantified by qPCR.  
26 The data are the average from three independent experiments.

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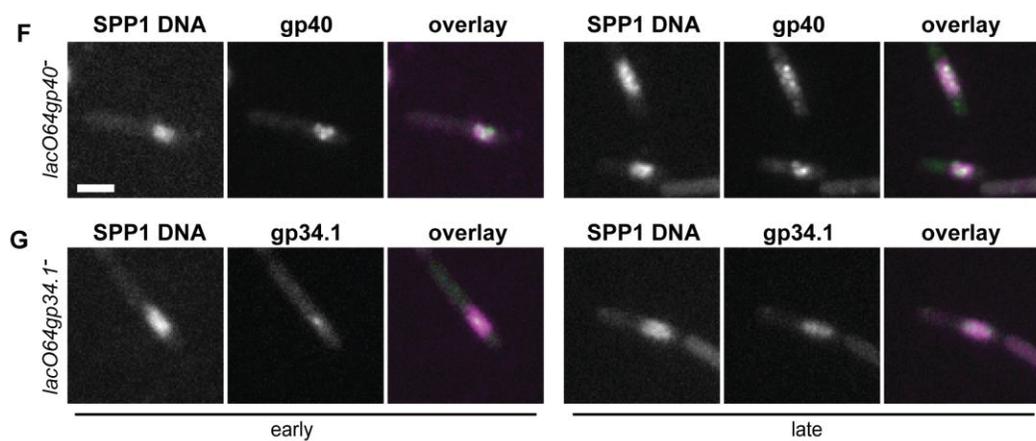
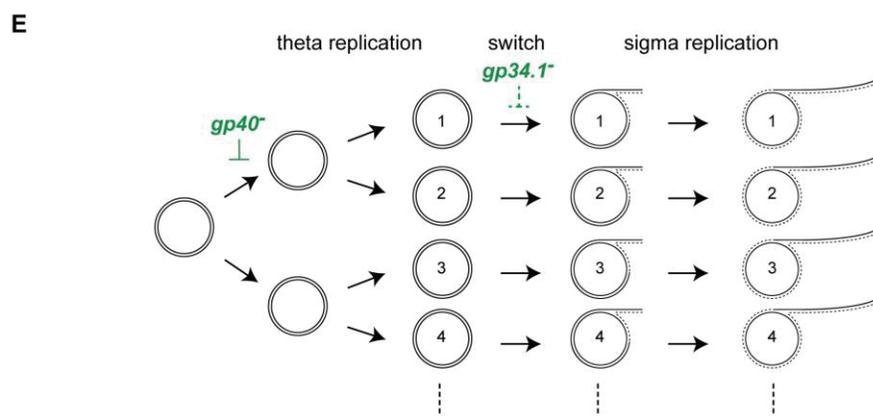
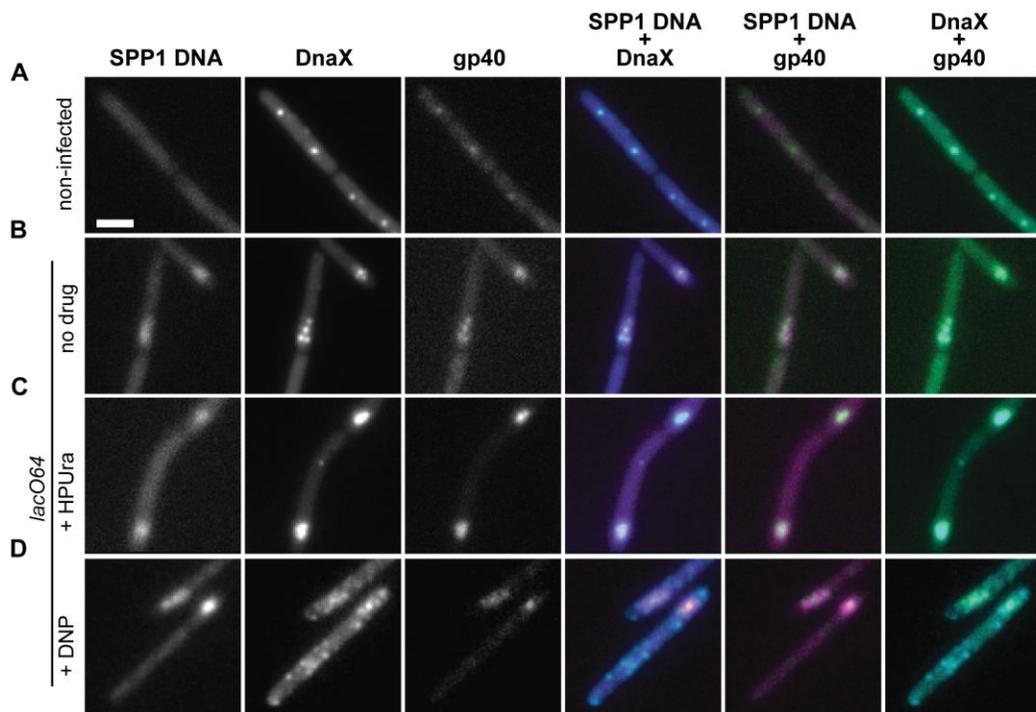
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**Fig. 2. Cellular localization of *B. subtilis* replisome proteins during SPP1 infection.**

(A) PolC-GFP (top) and mCFP-DnaX (bottom) in bacteria non-infected (left) and infected with SPP1/acO64 (right) late p.i (see *SI Appendix* Fig. S2B for experimental setup). The *B. subtilis* strains produce LacI-mCherry constitutively. Signals on the RFP channel are displayed in magenta while signals on GFP and CFP channels are in green on overlays, respectively. The scale bar represents 2  $\mu$ m. Arrowheads show the position of host replication protein foci in non-infected bacteria and the arrow identifies a focus of mCFP-DnaX that does not colocalize with SPP1 DNA in an infected bacterium. (B) Quantitative analysis of *B. subtilis* replisome proteins localization relative to the SPP1 DNA focus in bacteria early p.i. according to the grey scale legend on the right. More than 100 cell counts were made for each strain.



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45 **Fig. 3. Cellular localization of SPP1 proteins engaged in phage DNA replication (gp40 helicase)**  
46 **and in the switch of genome replication mode (gp34.1 5'-3' exonuclease).**

47 (A-D) Localization of SPP1 DNA, mCFP-DnaX (*B. subtilis* clamp loader subunit) and gp40-mCitrine in  
48 non-infected bacteria (A) and in SPP1/*lacO64* infected bacteria untreated (B), incubated with 200  $\mu$ M  
49 HPUra (C) or with 10 mM DNP (D). Drugs were added at 21 min p.i. to the infected culture that was  
50 spotted in the agarose pad containing the same concentration of drug 3 min later. Fluorescence  
51 microscopy acquisitions were made between 50 and 60 min p.i.. The scale bar in (A) represents 2  $\mu$ m.  
52 (E) Scheme of SPP1 replication initiated by a theta mode, leading to an exponential increase of  
53 independent circular genome molecules, followed by a switch to sigma replication that generates linear  
54 concatemers for DNA packaging. SPP1 mutants blocking replication initiation (*gp40*) or severely  
55 affecting the switch from theta to sigma mode (*gp34.1*) are displayed in green. (F,G) Co-localization of  
56 gp40-mCitrine (F) and gp34.1-mCitrine (G) with phage DNA in infections with SPP1 mutants defective in  
57 gp40 and in gp34.1 production (trans-complementation conditions), respectively. Images at early (left)  
58 and late (right) stages of infection (*SI Appendix Fig. S2B*) are displayed. The scale bar in (F) represents  
59 2  $\mu$ m.

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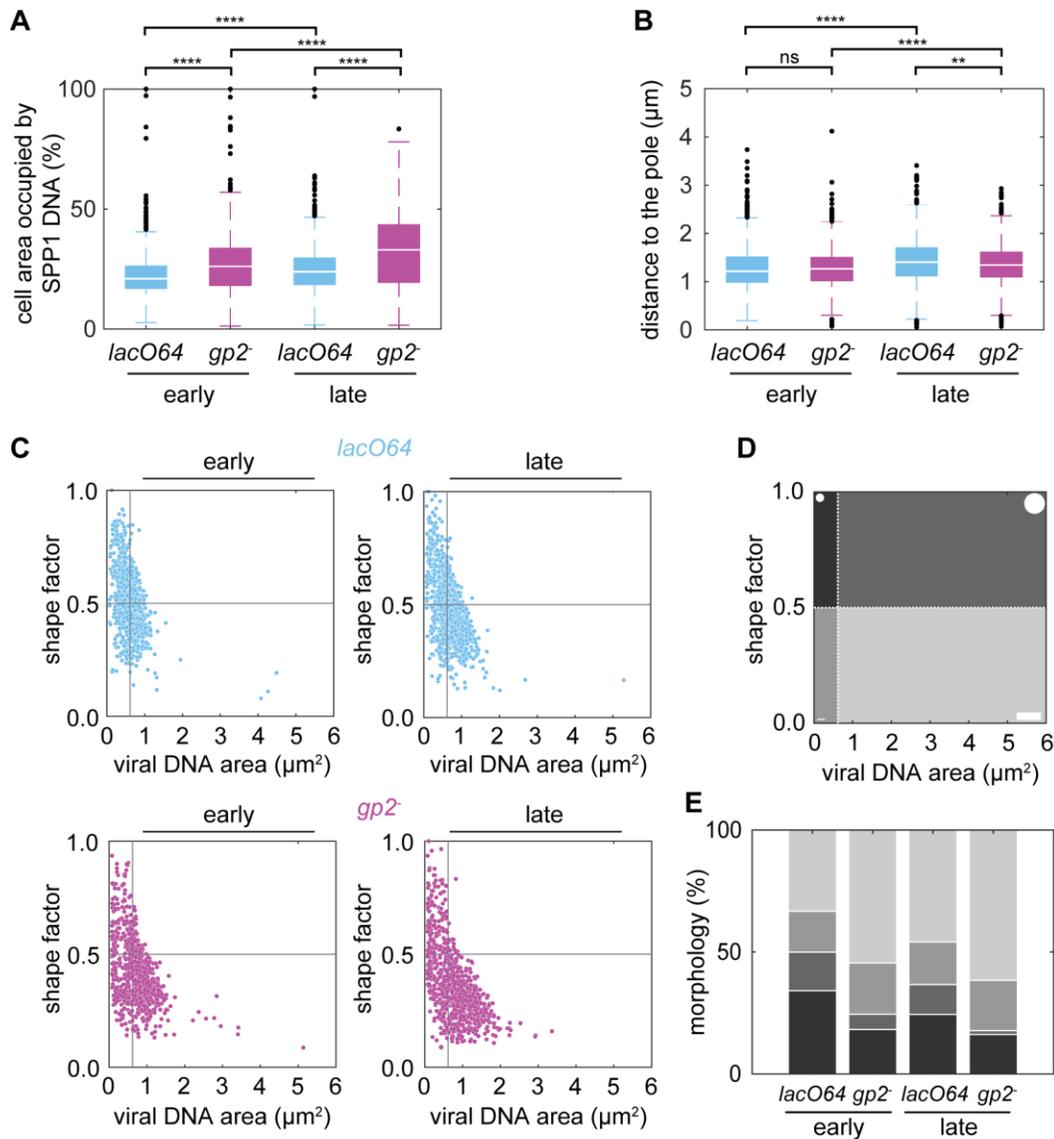
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74 **Fig. 4. Temporal evolution of the viral DNA compartment.**

75 (A) Percentage of cell area occupied by the SPP1 DNA focus in *B. subtilis* GSY10025 mono-infected  
 76 (one viral DNA focus per cell) with SPP1/*lacO64* (blue) or with the DNA packaging-defective mutant  
 77 SPP1/*lacO64gp2* (magenta) at early and late times p.i. (*SI Appendix Fig. S2B*). More than 800 cells from  
 78 three independent infections were analyzed. The same datasets were used for all analyses in the figure.

79 (B) Average distance of the SPP1 DNA focus center of mass to the proximal cell pole. Boxplots in (A)  
 80 and (B) show the median of the sample data as a white solid line and draws point as outliers if they are  
 81 greater than  $q_3 + w(q_3 - q_1)$  or less than  $q_1 - w(q_3 - q_1)$ , where  $q_1$  and  $q_3$  are the 25<sup>th</sup> and 75<sup>th</sup>  
 82 percentiles of the sample data, respectively, and  $w = 1.5$ . Statistical significance between conditions in  
 83 (A) and in (B) were calculated using the Mann–Whitney non-parametric test (\*\*\*\* =  $P < 0.0001$ ; \*\*\* =

84 0.0001<P<0.001; \*\* = 0.001<P<0.01; \* = 0.01<P<0.05; ns = P>0.05). (C) Surface aspect of the phage  
85 DNA focus. The width to length ratio (shape factor) of the segmented focus is plotted relative to its area.  
86 The vertical and horizontal limits separating all quadrants are defined, respectively, as the median values  
87 of the DNA surface and the shape factor from the dataset associated with the infection with SPP1 *lacO64*.  
88 Foci shape factors were quantified when DNA encapsidation took place (blue; infection with SPP1 *lacO64*,  
89 abbreviated *lacO64* in the figure) or was blocked (magenta; infection with SPP1 *lacO64gp2<sup>-</sup>*, abbreviated  
90 *gp2<sup>-</sup>* in the figure) at early (left) and late (right) times p.i.. (D) Schematic visual aid of the DNA focus shape  
91 observed in each quadrant of the plot in (C). (E) Percentage of viral DNA compartment shape types in  
92 the quadrants of (C) displayed according to the grey scale of (D).

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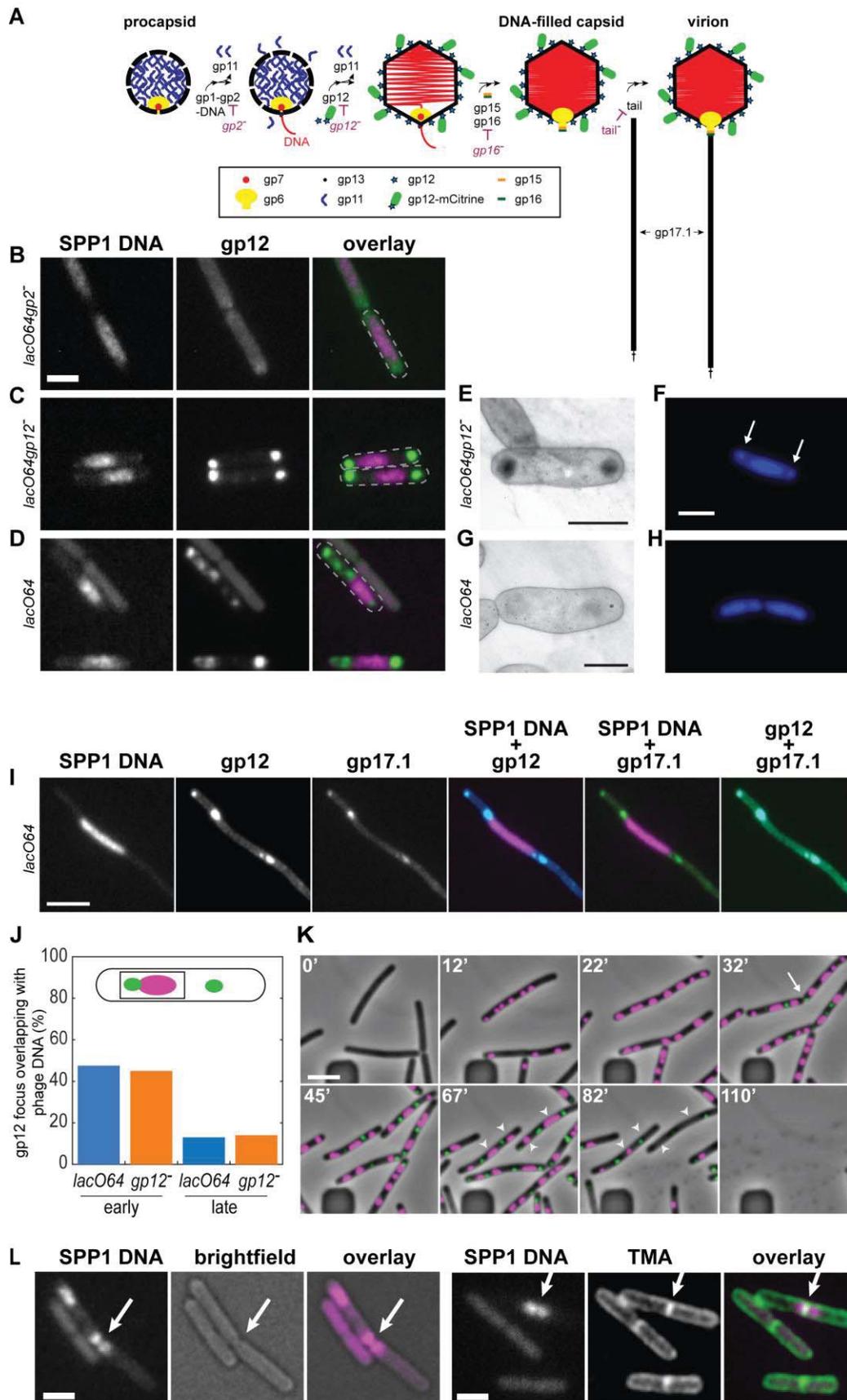
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113 colloidal gold, respectively. The scale bars represent 1  $\mu\text{m}$  in the EM sections. (G and H) Epifluorescence  
114 of fixed bacteria stained with DAPI from the cultures used for immunoEM experiments.  
115 (I and J) Cytological localization of LacI-mCherry that decorates SPP1 DNA (magenta in (J)) and of  
116 mCitrine-gp11 that is incorporated in procapsids (green in (J)) in *B. subtilis* GSY10025 non-infected and  
117 infected with SPP1/lacO64gp2<sup>-</sup>. Grey dotted lines display the cell outline defined from brightfield imaging.  
118 The scale bars represent 2  $\mu\text{m}$  in the fluorescence microscopy images of (G) and (I). (K) Scheme of  
119 procapsid formation and DNA packaging initiation steps during SPP1 viral particle assembly. All  
120 components of the SPP1 procapsid and of the DNA packaging apparatus are presented. The major  
121 capsid protein gp13 lattice is displayed as a dashed line delimiting the procapsid and by a thin icosahedral  
122 outline in the expanded capsid. MCitrine is displayed as a green cylinder in the mCitrine-gp11 fusion  
123 protein. Mutants, used in this study, that block synthesis of different SPP1 proteins engaged in procapsid  
124 assembly and DNA packaging are also presented. (L) Time-lapse of filamented *B. subtilis* GSY10025  
125 (producing mCitrine-gp11) infected with SPP1/lacO64gp2<sup>-</sup>. Cell division was inhibited 1 h before infection  
126 by addition of 1  $\mu\text{g}/\text{mL}$  PC190723. Signals on the RFP and YFP channels are displayed in magenta and  
127 green in overlays, respectively. Time p.i. is displayed on the right top corner of the overlay. The arrow  
128 shows dispersion of SPP1 DNA (magenta) in the agarose pad upon cell disruption at 85 min p.i. while  
129 clusters of procapsids (green) maintain their integrity. The scale bar represents 5  $\mu\text{m}$ . (M) Infection of  
130 filamented *B. subtilis* GSY10025 with SPP1/lacO64gp6<sup>-</sup> defective in production of the procapsid portal  
131 protein. Portal-less procapsids assembled during infection are not competent to package viral DNA (42).  
132 An infected cell late p.i. is presented surrounded by non-infected bacteria. Experimental conditions are  
133 as in (L).

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145 **Fig. 6. Localization of SPP1 DNA and DNA-filled particles in infected bacteria.**  
146 (A) Scheme of the SPP1 viral particle assembly pathway. MCitrine is displayed as a green cylinder in the  
147 gp12-mCitrine fusion protein. (B-D) Localization of SPP1 DNA and of capsid auxiliary protein gp12-  
148 mCitrine that decorates DNA-filled particles in *B. subtilis* GSY10024 infected with SPP1*lacO64gp2<sup>-</sup>* (B),  
149 SPP1*lacO64gp12<sup>-</sup>* (C), and SPP1*lacO64* (D). Images at late stages of infection (SI Appendix Fig. S2B)  
150 are displayed. Grey dotted lines display cell outlines defined from brightfield imaging. The scale bar  
151 represents 2  $\mu\text{m}$ . (E and G) Thin sections of *B. subtilis* YB886 infected with SPP1*lacO64gp12<sup>-</sup>* (E) and  
152 SPP1*lacO64* (G) immunolabeled as in Fig. 5 A and B. The scale bars represent 1  $\mu\text{m}$ . (F and H) DAPI-  
153 staining of fixed bacteria from the cultures used for immunoEM experiments. Arrows in (F) identify  
154 individualized DAPI foci attributed to warehouses. The scale bar in (F) represents 2  $\mu\text{m}$ . (I) Infection of  
155 *B. subtilis* GSY10096 filamented cells with SPP1*lacO64*. The fluorescent reporters of SPP1 DNA,  
156 capsids, and tails were LacI-mCherry (magenta in overlays), gp12-mCFP (blue) and gp17.1-mCitrine  
157 (green), respectively. Images were taken late p.i.. The scale bar represents 5  $\mu\text{m}$ . (J) Quantification of  
158 DNA-filled particles overlapping with the phage DNA compartment of cells mono-infected with  
159 SPP1*lacO64* and SPP1*lacO64gp12<sup>-</sup>* at early and late times p.i.. Datasets of >500 cells were analysed.  
160 (K) Snapshots from time-lapse Movie S1 of *B. subtilis* GSY10024, producing gp12-mCitrine, immobilized  
161 in a CellAsic microfluidics system and infected with SPP1*lacO64gp12<sup>-</sup>*. The first photo shows cells before  
162 infection followed by snapshots at time points indicated on the top left corner. They illustrate the temporal  
163 and spatial program of SPP1 DNA compartment and viral particles warehouses formation (displayed in  
164 magenta and green respectively). Cells are multi-infected. The white arrow identifies a bacterium  
165 undergoing division. Arrowheads show the position of DNA compartments at 67 min p.i. that are  
166 disassembled in cell ghosts at 82 min p.i. but prior to full bacterial lysis (110 min p.i.). Images were  
167 enhanced as described in Movie S1 legend. (L) Cell septation through the SPP1 DNA compartment  
168 (displayed in magenta) in bacteria producing LacI-mCherry (strain GSY10004) infected with  
169 SPP1*lacO64*. Cells membrane contour is stained with TMA-DPH (rendered in green) on the right side.  
170 The white arrow shows septum formation. To enhance the signal from the membrane staining (TMA-  
171 DPH), the raw image was denoised (2D-Gaussian filter, radius = 1,25 pixel) and background was  
172 removed using a rolling ball (sigma = 10 pixel) using Fiji (67).

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## Supplementary Information for

### Temporal Compartmentalization of Viral Infection in Bacterial Cells

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#### This PDF file includes:

Materials and Methods

Figures S1 to S5

Tables S1 to S4

High resolution electron micrographs of Figs. 5A, 5B, 5C, 5D, 5E, 5F, 6E and 6G

Legend for Movie S1

Legend for Dataset S1

SI References

#### Other supplementary materials for this manuscript include the following:

Movie S1

Dataset 1

## Materials and Methods

**Bacterial strains construction.** Bacterial strains used in this study are listed in Table S3.

*B. subtilis* GSY10004 was constructed by transformation of *B. subtilis* YB886 with plasmid pPT300 linearized with XbaI. *B. subtilis* GSY10024, GSY10025, BG1829, BG1831, BG1833, BG1837, GSY10031, GSY10060 and GSY10095 were constructed by transformation of GSY10004 with plasmids pAL24, pAL25, pML1829, pML1831, pML1833, pML1839, pAL31, pAL36 and pAL53, respectively, linearized with ScaI. *B. subtilis* GSY10006, GSY10008, GSY10010, GSY10011, GSY10012 and GSY10014 were constructed by transformation of GSY10004 with chromosomal DNA from *B. subtilis* strains PolC-GFP, GFP-DnaN, GFP-SsbA, GFP-DnaB, DnaE-GFP and GFP-DnaC, respectively. GSY100050 was constructed by transformation of GSY10000 with plasmid pHM232. GSY100051 was constructed by transformation of GSY10050 with chromosomal DNA from strain HM1049. GSY10061 was constructed by transformation of GSY10060 with plasmid pML1829 linearized with ScaI. GSY10096 was constructed by transformation of GSY10095 with plasmid pAL44 linearized with ScaI.

Transformation of competent *B. subtilis* cells was performed by a two-step starvation protocol using Spizizen medium for growth (1). 1 µg of linearized plasmid or chromosomal DNA was added to 1 mL competent cells for 60 min at 37°C, plated onto appropriate selective plates, and incubated overnight at 37°C.

**Growth conditions.** *E. coli* and *B. subtilis* strains were grown in Luria-Bertani (LB) medium, unless stated otherwise. Antibiotics selection in *E. coli* were 100 µg/mL ampicillin and 30 µg/mL erythromycin. Antibiotics selection in *B. subtilis* were 5 µg/mL kanamycin, 0.5 µg/mL erythromycin and 12.5 µg/mL lincomycin (*mls* resistance), 100 µg/mL spectinomycin, and 5 µg/mL neomycin. *B. subtilis* BG295 and HA101B strains were cultivated in MIII minimal medium (2) supplemented with 50 µg/mL tryptophan for BG295 and 50 µg/mL tryptophan, 50 µg/mL leucine for HA101B.

**Phage strains construction.** Phages used in the present study are listed in Table S3. Multiple SPP1 mutants were produced by phage crosses. The two parental phages were used to infect a permissive strain in LB medium supplemented with 10 mM CaCl<sub>2</sub> at an input multiplicity of 10 pfu/cfu of each phage for 2 h at 37°C. The cell lysate was cleared by centrifugation at 12,000 g for 20 min at 4°C and titrated on a permissive strain. Isolated phage plaques were collected and resuspended in 200 µL TBT buffer (100 mM Tris-Cl pH 7.5, 100 mM NaCl, 10 mM MgCl<sub>2</sub>) on 96-well plates. The phage clones genotype was screened initially by spot test on complementing strains. Multiple mutant candidates were then checked by titration on strains complementing or rescuing the phage *sus* mutation knock-out and by PCR for deletion and insertion mutations.

**Plasmids construction.** Plasmids and primers used in the present study are listed in Table S3.

pPT300 was created in two steps. First, pAL1 was generated by cloning a PCR product of *mcherry* amplified from vector pJ1 with primers 1 and 2 cut with XhoI and BamHI and ligated into plasmid pHP13 cut with the same restriction enzymes. Second, pPT300 was generated by digestion of pAL1 with EcoRI and BamHI and cloning of the fragment carrying *mcherry* in pKL190 cut with the same enzymes.

pAL24, pAL53, pML1837, pML1833, and pML1829 were constructed in three steps. First, pAL19 was generated by ligation of a PCR product coding  $P_{spac}$ , amplified from plasmid pDH88 with primers 3 and 4 cut with BamHI-XbaI to pSac-Kan cut with BglII-XbaI. Then, pAL21 was generated by ligation of a PCR product coding *mcitrine*, amplified from pJexpress404 with primers 5 and 6 cut with with EcoRI-BamHI to pAL19 cut with the same enzymes. Finally, pAL24, pAL53, pML1837, pML1833, and pML1829 were generated by ligation of a PCR product of SPP1 genes *12*, *17.1*, *34.1*, *35*, and *40* (amplified from SPP1 DNA with primers 7 and 8, 9 and 10, 11 and 12, 13 and 14, and 15 and 16, respectively), cut with ClaI-XhoI to pAL21 cut with the same enzymes.

pAL25 and pML1831 were constructed in two steps. First, pAL20 was generated by digestion of pJexpress404 with EcoRI-BamHI and ligation of the fragment coding *mcitrine* to pAL19 cut with the same enzymes. Second, pAL25 and pML1831 were generated by ligation of a PCR product coding genes *11* and *35* (amplified from SPP1 DNA with primers 17 and 18, and 13 and 19, respectively) cut with ClaI-BamHI to pAL20 cut with the same enzymes.

pAL36 and pAL37 were constructed in two steps. First, pAL28 was generated by ligation of a PCR product coding *mcfp*, amplified from pDR200 with primers 20 and 21, cut with BamHI-KpnI to pDG1190 cut with the same enzymes. Second, pAL36 and pAL37 were generated by ligation of PCR products coding *dnaG* or *dnaX* (amplified from *B. subtilis* DNA with primers 22 and 23, 24 and 25, respectively), cut with BamHI-ClaI to pAL28 cut with the same enzymes.

pAL44 was constructed in two steps. First, pAL29 was generated by ligation of a PCR product coding *mcfp*, amplified from pDR200 with primers 23 and 24, cut with EcoRI-SpeI to pDG1192 cut with the same enzymes. Second, pAL44 was generated by ligation of a PCR product coding gene *12*, amplified from SPP1 DNA with primers 28 and 29, cut with KpnI-XhoI to pAL29 cut with the same enzymes.

Plasmid constructions were transformed, selected with appropriate antibiotic concentrations (see sections Bacterial strains construction and Growth conditions) and amplified in *E. coli* DH5 $\alpha$  using standard molecular biology protocols (3). Correctness of all constructs was confirmed by DNA sequencing.

**Phage amplification.** Phages SPP1*wt*, *delX110*, *lacO64* and *lacO64gp12*<sup>-</sup> were multiplied in *B. subtilis* YB886 as described (4). Phages SPP1*lacO64gp2*<sup>-</sup>, *lacO64gp6*<sup>-</sup>, *lacO64gp11*<sup>-</sup>, *lacO64gp13*<sup>-</sup>, and *lacO64gp16*<sup>-</sup> were multiplied in the permissive strain *B. subtilis* HA101B while *lacO64gp34.1*<sup>-</sup>, *lacO64gp40*<sup>-</sup>, and *lacO64tail*<sup>-</sup> were multiplied in the permissive host *B. subtilis* BG295, as described (5, 6). The *lacO* operators cassette was sequenced in SPP1*lacO64* and its stable presence was monitored by PCR during amplification of all phage strains (4). The *lacO* repeats are flanked by 10 bp random sequences to prevent recombination within the *lacO* array to ensure its genetic stability (7).

**Quantification of intracellular phages.** Aliquots of 100 µL of infected cells were taken at the desired time points p.i., diluted to 1 mL with TBT containing 10% chloroform and vortexed vigorously. After 2 h on ice to allow for complete cell lysis, the mix was centrifuged, and the aqueous phase was titrated to determine the intracellular phages during the infection cycle.

**Purification of SPP1 particles.** SPP1 procapsids and phages were produced and partially purified using a protocol adapted from (8). In brief, 300 mL cultures of *B. subtilis* GSY10004, GSY10024 and GSY10025, supplemented with 1 mM IPTG for GSY10024 and GSY10025, were infected at an OD<sub>600nm</sub> of 0.8 with different SPP1 phage mutants at an input multiplicity of 5 pfu/cfu and incubated with orbital shaking at 37°C for 3 h. Cultures were centrifuged at 15,300 g for 15 min at 4°C to sediment intact bacteria and debris. SPP1 particles in the supernatants were sedimented by centrifugation overnight at 20,300 g (4°C). Supernatants were fully removed and pellets were covered with 250 µL of cold buffer R (50 mM HEPES-KOH pH 7.6, 50 mM potassium glutamate, and with a 1X protease inhibitors cocktail [Roche]) supplemented with 10 mM EDTA for procapsids purification. After incubation for 15 minutes at 4°C, pellets were resuspended very carefully, supplemented with 25 mM MgCl<sub>2</sub>, 25 U Benzonase (Millipore), 100 µg/mL RNase, and incubated for 3 h at 4°C in a rotating wheel. Debris were removed by centrifugation for 15 min at 25,900 g (4°C). Supernatants (200 µL) were applied to a 10-30% glycerol gradient (v/v) in buffer R and centrifuged for 90 min at 35,000 rpm (4°C). Fractions of 1 mL were collected and analyzed on 12% SDS-PAGE gels stained with Coomassie Blue. Fractions with SPP1 particles (procapsids, capsids or virions) were pooled.

To obtain highly purified SPP1 virions, lysates of infected bacteria were sedimented overnight at 12,000 g (4°C), resuspended in TBT buffer, and run for 6 h at 35 Krpm in a SW41 rotor (Beckman) (20°C) through a discontinuous CsCl gradient in TBT buffer (4).

Viral particles were applied to 12% SDS-PAGE gels and their composition was analysed by Coomassie Blue staining and western blotting with anti-gp11 or anti-GFP rabbit polyclonal sera (Table S3).

**Preparation of cell lysates and protein composition analysis.** Strains YB886, GSY10004, GSY10024 and GSY10025 were grown overnight at 30°C in LB medium. Cultures were diluted 1:100 in 25 mL of LB medium (containing 1 mM IPTG for GSY10024 and GSY10025) and grown at 37°C. At an OD<sub>600nm</sub> of 0.8, cultures were supplemented with 10 mM CaCl<sub>2</sub> and infected with different phages at an input multiplicity of 5 pfu/cfu. At 25 min p.i., bacteria were centrifuged for 5 min at 8,000 g (4°C). Cell pellets were resuspended in 1:20 of the initial culture volume in 50 mM glucose, 1 mM EDTA, 50 mM Tris-HCl pH 8, 1 mg/mL lysozyme and 1X protease inhibitors cocktail for 5 min at room temperature. An equal volume of ice-cold lysis buffer containing 500 mM NaCl, 1% NP-40, 50 mM Tris-HCl pH 8, 5 mM MgCl<sub>2</sub>, 1 µg/mL DNase and 100 µg/mL RNase was added and the mix was kept on ice for 30 min. Cell debris were removed by centrifugation for 30 minutes at 14,000 g (4°C) and supernatants were immediately run on a 12% SDS-PAGE gel. Protein composition was determined as described in the previous section. This protocol was adapted from (9).

**Quantitative PCR (qPCR) experiments.** An overnight culture of *B. subtilis* GSY10004 strain was diluted 1:100 in fresh LB medium and grown at 37°C to an OD<sub>600</sub> of 0.8. The culture was supplemented with 10 mM CaCl<sub>2</sub> and infected at an input multiplicity of 5 pfu/cfu with phages SPP1 *lacO64*, *lacO64gp2<sup>-</sup>* or *lacO64gp40<sup>-</sup>*. Two aliquots of 1 mL culture were taken for each time point and for each cell treatment procedure (technical duplicates). Samples were centrifuged 3 min at 11,000 g. The supernatant was completely removed to eliminate extracellular phage particles and cell pellets were frozen at -20°C. Pellets were resuspended in 500 µL lysis buffer (50 mM Tris-HCl pH 8, 50 mM glucose, 50 mM NaCl, 2 mg/mL lysozyme and 1X protease inhibitors cocktail) and incubated for 10 min at room temperature. For total DNA extracts, the cell suspension was mixed with 500 µL lysis buffer A (50 mM Tris-HCl pH 8, 50 mM NaCl, 1% NP40, 10 mM EDTA and 10 µg/mL RNase) and incubated for 30 min on ice. For DNase-treated extracts, the cell suspension was mixed with 500 µL of lysis buffer B (50 mM Tris-HCl pH 8, 50 mM NaCl, 1% NP40, 5 mM MgCl<sub>2</sub>, 100 µg/mL RNase and 100 U Benzonase), incubated for 30 minutes at 37°C and supplemented with 10 mM EDTA. Both types of extracts were deproteinized with 0.5% SDS and 50 µg/mL proteinase K (Roche) for 1 h at 65°C. Biological duplicates were prepared for each condition and qPCR experiments were carried out for three series of samples from independent infection experiments.

Quantitative PCR was performed on a QuantStudio 12K Flex Real-Time PCR System with a SYBR green detection protocol (Life Technologies) following the manufacturer's instructions. To prepare the samples, the DNA extracts were first diluted 300-fold with water. 3  $\mu$ L of diluted samples were then mixed with Fast SYBR Green Master Mix and 500 nM of each primer for a final volume of 10  $\mu$ L. The reaction mixture was loaded on 384 well microplates and submitted to 40 cycles of PCR (95°C/20 s; [95°C/1 s; 60°C/20 s] x 40) followed by a fusion cycle to analyze the melting curve of the PCR products. All the qPCR reactions were made in technical duplicates. A negative control, without added sample, was included in each experiment. Primers were designed using the Primer-Blast tool from NCBI and the Primer Express 3.0 software (Life Technologies) to quantify SPP1 gene 6 and *B. subtilis* gene *gyrA* (Table S3). Specificity and the absence of multilocus matching at the primer site were verified by BLAST analysis on the genomes of SPP1 and *B. subtilis*.

Absolute quantification of the copy number from each target gene in the samples was obtained using a standard curve. The curve was prepared by a five-fold dilution series of linearized plasmid pUC19-EJ1 containing DNA sequences of the SPP1 and *B. subtilis* amplicons. The known quantity of plasmid DNA copies in the standard curve from  $1 \times 10^7$  to  $6.4 \times 10^2$  copies, allowed converting Ct values determined experimentally into the copy number for each targeted gene. These copy numbers of SPP1 and *B. subtilis* genomes present in a sample were then used to calculate the number of SPP1 phages per bacterium considering that there are an average of 2.7 copies of *B. subtilis* genomes per cell (*SI Appendix* Fig. S1 C and D). The number of SPP1 genomes protected inside viral particles was quantified in samples treated with DNase (see above) and divided by the total number of SPP1 genomes in the sample to calculate the percentage of encapsidated SPP1.

**Total DNA quantification by Qubit.** Deproteinized DNA samples were prepared as described for qPCR experiments (previous section). Qubit quantifications were carried out using a Qubit™ dsDNA HS Assay kit in a Qubit™ 3.0 Fluorometer. All measurements were made in a final volume of 200  $\mu$ L Qubit dsDNA HS buffer with 1  $\mu$ L of fluorophore reagent and 3  $\mu$ L of buffer or sample used for extraction of total DNA extracts. A standard curve was prepared with increasing quantities of the kit DNA HS Standard 2. Each sample was prepared in duplicate with 3  $\mu$ L of extract and was measured twice. The DNA concentration of each sample was determined according the standard range equation and the average of the 4 measurements.

**Fluorescence Microscopy.** Overnight cultures of *B. subtilis* strains grown at 30°C were diluted 1:100 in fresh LB, pH 8 medium with 1 mM IPTG or/and 1% xylose, depending on the strain, and grown at 37°C.

At an  $OD_{600nm}$  of 0.8 the cultures were supplemented with 10 mM  $CaCl_2$  and infected with the SPP1 strain under analysis at an input multiplicity of 1. Infected cultures were grown at 37°C with orbital shaking for 12-20 min, according to the experimental setup outlined in *SI Appendix Fig. S2B*. Infected cells were mounted on agarose pads and microscopy was carried out subsequently at room temperature. Image acquisitions were performed at early (20-30 min p.i.) and late (40-50 min p.i.) time-points after phage infection (*SI Appendix Fig. S2B*) on a Zeiss Axio Observer Z1 microscope with 63X oil objective. We noted that LacI-mCherry remained associated to the *lacO64* repeats on SPP1 DNA in presence of 1 mM IPTG.

HPUra (a gift from George Wright and Neal Brown, Glsynthesis, Worcester, USA) (200  $\mu$ M final concentration) and DNP (Sigma Aldrich) (10 mM) were added to cultures at 21 min p.i., incubated for 3 min at room temperature and mounted on agarose pads for epifluorescence microscopy. HPUra and DNP were also added in the agarose pad at the same final concentration.

DAPI (Thermo Fisher Scientific) (10 mg/mL) and TMA-DPH (Thermo Fisher Scientific) (25  $\mu$ M) were added to cultures for 3 min at room temperature before observation and mounted on agarose pads for epifluorescence microscopy.

**Filamentated cells infection and imaging.** Overnight cultures of *B. subtilis* were diluted 1:100 in fresh LB medium supplemented with 1 mM IPTG or/and 1% xylose and grown at 37°C. At an  $OD_{600nm}$  ~0.4-0.5, PC190723 (FstZ Polymer Stabilizer, Merck-Millipore) was added to a final concentration of 1  $\mu$ g/mL and the cultures were further incubated for 60 min at 37°C. Then, cells were supplemented with 10 mM  $CaCl_2$  and infected with the SPP1 strain to be tested for 20 min at 37°C. The input multiplicity of infection was adjusted empirically to obtain <50% infected cells. Cells were mounted in agarose pads and imaged as described in the previous section. Image acquisitions were performed every 10 min for GSY10025 and every 5 min for GSY10096.

**Microfluidics experiments.** An overnight culture of *B. subtilis* GSY10024 grown on LB medium was diluted 1:100 in fresh LB medium and incubated with agitation at 37°C. At an  $OD_{600nm}$  of ~0.4-0.5, cells were concentrated 5-fold and loaded into B04A microfluidic plates (ONIX, CellASIC ONIX Microfluidic Plates for Bacterial cells) at 2 psi for 12 s. The medium flow rate and temperature were maintained at 5 psi and 37°C, respectively, throughout imaging. The loading channel was subsequently washed for 20 min with LB medium supplemented with 10 mM  $CaCl_2$ . Phage was added to the cells for 5 min by switching the media flow channel using CellASIC ONIX FG (Version 5.0.2-Millipore). After phage infection, cells were visualized using a Nikon Ti-Eclipse microscope, stabilized at 37°C, with a 100X oil

objective (NA 1.3 WD : 0,2 mm CFI Plan Fluor - Phase - Nikon). Images were taken every 2.5 min using red and yellow fluorescence. Images and videos were processed as described in the legend of Movie S1.

**Electron microscopy of cryo-sectioned infected cells.** An overnight culture of *B. subtilis* YB886 was diluted 1:100 in fresh LB medium and grown at 37°C to an OD<sub>600nm</sub> of 0.8. The culture was supplemented with 10 mM CaCl<sub>2</sub> and infected at a multiplicity of 5 pfu/cfu with phages SPP1*lacO64*, *lacO64gp2<sup>-</sup>* or *lacO64gp12<sup>-</sup>* at 37°C with agitation. At 35 min p.i., cells were mixed with fixation solution (4% paraformaldehyde [Electron Microscopy Sciences], 0.4% glutaraldehyde [Sigma Aldrich], 20 mM MgCl<sub>2</sub>, 1X PBS pH 8) at room temperature for 2 h on a rotating wheel. Fixed cells were centrifuged for 10 min at 3,500 g, washed twice with wash buffer (10 mM MgCl<sub>2</sub>, 1X PBS pH 8), and resuspended in TBT buffer. Cells were centrifuged and the supernatant carefully removed. Pellets were embedded in 10% gelatin dissolved in TBT. Cells embedded in gelatin beads were conserved in 2.3 M sucrose prepared in TBT at 4°C. Beads were cut in 90 nm thin sections at cryo-temperatures with an EM UC7 ultramicrotome under liquid nitrogen (10–12).

Sections were transferred to copper grids covered with carbon film, washed 5 times in TBT and incubated in buffer A (Table S4) for 1 h at room temperature. Grids were washed 3 times with TBT, twice with buffer B (1X PBS pH 8 supplemented with 10 mM CaCl<sub>2</sub>), blocked with 500 µL blocking buffer for 30 min at room temperature, and incubated with 10 µL of primary antibody at 4°C (Tables S3 and S4). After 4 washes with buffer B, the grids were incubated with 10 µL of secondary antibody labelled with colloidal gold beads (Tables S3 and S4) for 2 h in a humidity chamber. They were then washed 4 times in buffer B, twice in water, and plunged in a solution of 2% uranyl acetate for 30 s. Thin-sections were imaged on a FEI Tecnai G<sup>2</sup> F20 electron microscope.

**Automatic detection and quantification of viral compartments.** Automatic detection and characterization of phage DNA compartments (LacI-mCherry), procapsids (mCitrine-gp11) and virion warehouses (gp12-mCitrine) were performed using a Matlab-based custom algorithm (Matlab R2016B version). The analysis workflow can be decomposed in three main successive steps:

1. Cell detection and identification. This step aims to generate a binary image in which cells are individualized. First, pixels associated to background were identified by applying k-means method (with 2 kernels) on the epi-fluorescence image of viral (pro-)capsids. The corresponding bright-field image was sequentially processed to (i) emphasize cells from the background (by applying a 'top-hat' filter using a disk with 15 pixels radius as structuring element), (ii) enhance the cell contour (by a 'difference of Gaussian' filter) and (iii) assign all background pixels to a unique value (lower than any pixel in the image).

By applying the k-means method (with 3 kernels) on this processed image, pixels were then classified within three groups: background, cell outline and cell inside. The binary mask of the cells was obtained by keeping only pixels associated to the inside of the cells. The absence of membrane staining in our images prevented to isolate and separate all cells without error (particularly when cells form chains). Therefore, visual inspection of each binary mask was performed to correct for cell separations (when it was obvious on bright-field image) and/or to eliminate cells for which segmentation failed. Finally, connected pixels were detected in each binary mask, allowing to generate a labeled image displaying individual bacteria in which morphological features of the cells (surface and length) were quantified.

2. Localization and morphology of the phage DNA compartment. First, the epi-fluorescence image of phage DNA was pre-processed (denoising by a 3x3 pixels median filter) and background pixels (detected on step 1) were all assigned to a unique value (lower than any value in the image). Then, the DNA foci were segmented using the k-means method (with 3 kernels) by keeping only areas greater than 7 pixels<sup>2</sup>. The resulting binary image allowed to (i) identify, locate and measure the replication area, (ii) count replication foci per cell to identify non-infected, mono-infected or multi-infected cells, (iii) characterize the dimensions (length and width) of replication foci by fitting an ellipse around these zones, (iv) measure the distance from the center of the replication foci to the two poles of the cell, and (v) quantify the average intensity of the fluorescence in the replication zone.

3. Localization and morphology of procapsids and virion warehouses. Detection was performed only on mono-infected cells with two different approaches for procapsids and capsids foci. For viral procapsids, segmentation of mCitrine-gp11 foci was achieved by the k-means method (with 2 kernels), and by removing areas smaller than 6 pixels<sup>2</sup> and areas displaying less than 5% pixels with intensity above an activation threshold. The activation threshold was defined on the fluorescence intensity of non-infected cells as the average +3 standard deviation. For viral capsids, gp12-mCitrine foci were enhanced by subtraction of their own filtered images obtained by denoising (5x5 pixels 2D-median filter). Noise was removed using a grayscale morphological opening (using a disk with 2 pixel radius as structuring element). Then all pixels not associated to mono-infected cells were set to 'Not-a-Number' (NaN) value in the resulting image, and segmentation of capsid foci was achieved by the k-means method (with 2 kernels). To remove false detections, capsid signals displaying an intensity below the threshold (defined as the value presenting equal probability distributions for intensity associated to cytoplasm and capsids areas) and smaller than 6 pixels<sup>2</sup> were removed. For both procapsids and capsids, the resulting binary image allowed to (i) identify, count, locate and measure the area of foci per mono-infected cell, (ii) measure the distance between the (pro)capsid and the phage DNA compartment, (iii) measure the percentage of overlapping area between the (pro)capsids and the DNA compartment (in case of overlap) or the gap distance between the two areas (in case of non overlap), (iv) determine the side localization

of viral (pro)capsids in the cell as compared to phage DNA compartment, (v) characterize the morphology (length and width) of the compartment by adjusting an ellipse around these areas, and (iv) quantify their average fluorescence intensity.

**Image processing used for visualization.** Fig. 6K and Movie S1. The time-lapse was processed to highlight the phage DNA compartment and virion warehouses. Firstly, temporal lateral drift was estimated using the phase contrast channel and corrected on all channels (fluorescence and phase contrast). SPP1 DNA images were pre-processed to remove background signal (rolling ball subtraction, radius = 20 pixels) and enhanced using a Laplacian of Gaussian filter (with an increasing radius from 3 to 5 pixels to respect foci area enlargement during infection). Processed images were segmented using thresholding, converted to binary mask and blurred with a Gaussian filter (radius = 2 pixels) for visualization. Capsid warehouses were denoised using median filter (radius: 1 pixel), and enhanced using Laplacian of Gaussian filter (radius = 2 pixels). As soon as capsids appeared in the time-lapse they were segmented using thresholding, converted to binary mask after rejecting large foci ( $> 0.5 \mu\text{m}^2$ ) to remove false detections (based on results from Fig. 4C), and blurred with a Gaussian filter (radius = 2 pixels) for the visualization.

For Fig. 6L, to enhance the signal from the membrane staining (TMA), the raw image was denoised (2D-Gaussian filter, radius = 1.25 pixel) and background was removed using a rolling ball (sigma = 10 pixel) using Fiji (13).

**Multiplicity of infection quantification.** Multiplicity of infection (MOI) was calculated from microscopy acquisitions of SPP1 DNA (LacI-mCherry) assuming that the fraction of cells containing virus particles is given by a Poisson distribution:

$$P(n) = \frac{m^n e^{-m}}{n!}$$

where  $m$  is the multiplicity of infection,  $n$  is the number of infectious agents that enter the host cell, and  $P(n)$  is the probability that a cell will get infected by  $n$  bacteriophages. For non-infected cells ( $n=0$ ), the ratio or probability can be simplified as  $P(0) = \exp(-m)$  allowing to quantify easily the MOI as

$$m = -\log\left(\frac{N_{ni}}{N_t}\right)$$

where  $N_{ni}$  is the number of non-infected cells and  $N_t$  is the total number of cells.

**Statistical analysis.** All statistical tests were performed using GraphPad Prism 6.05 and a non-parametric statistical test (two-tailed Mann-Whitney test with an alpha level of 5%).

Supplementary Figures

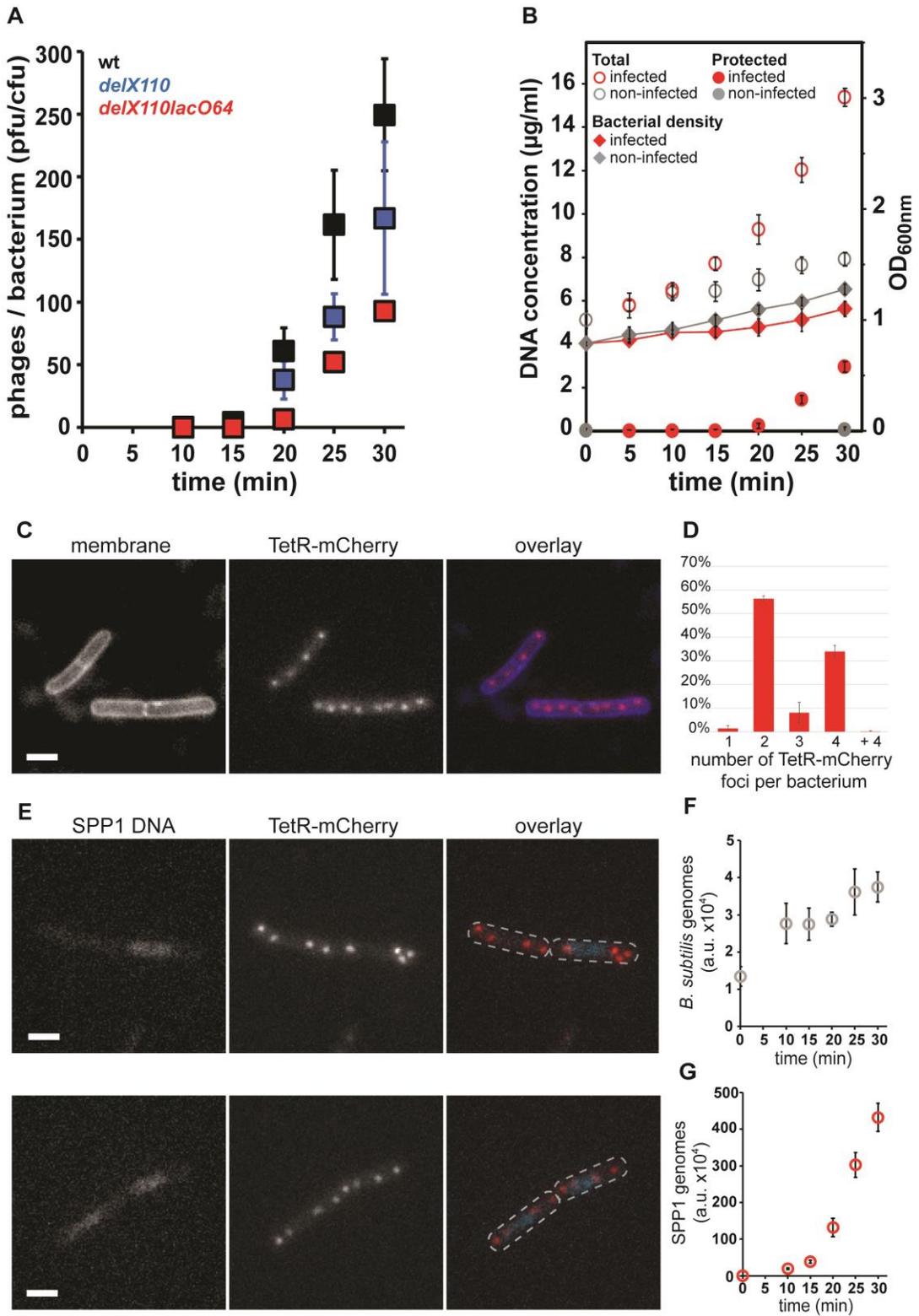
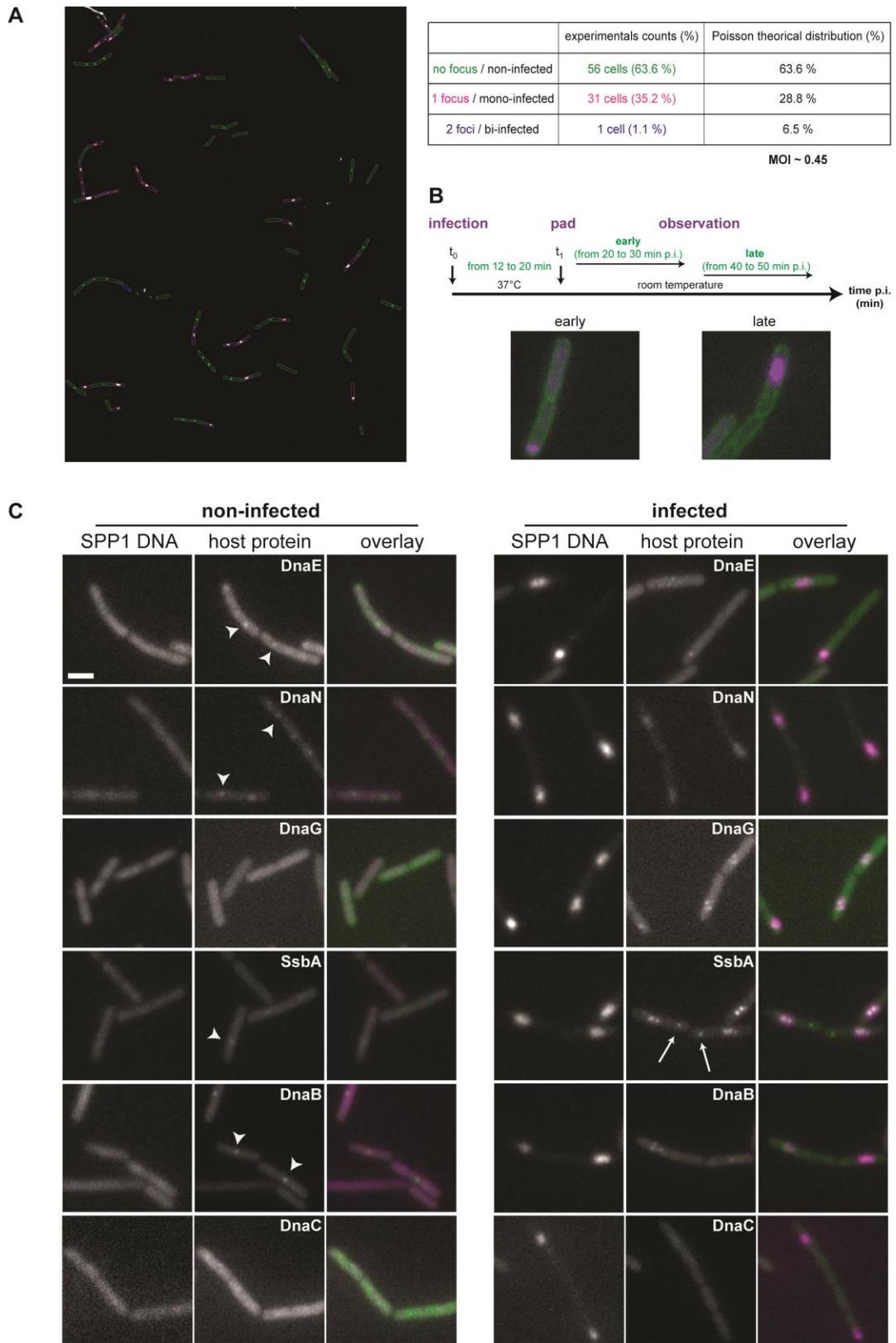


Fig. S1

**Fig. S1: Phage yield and DNA replication in SPP1-infected bacteria.**

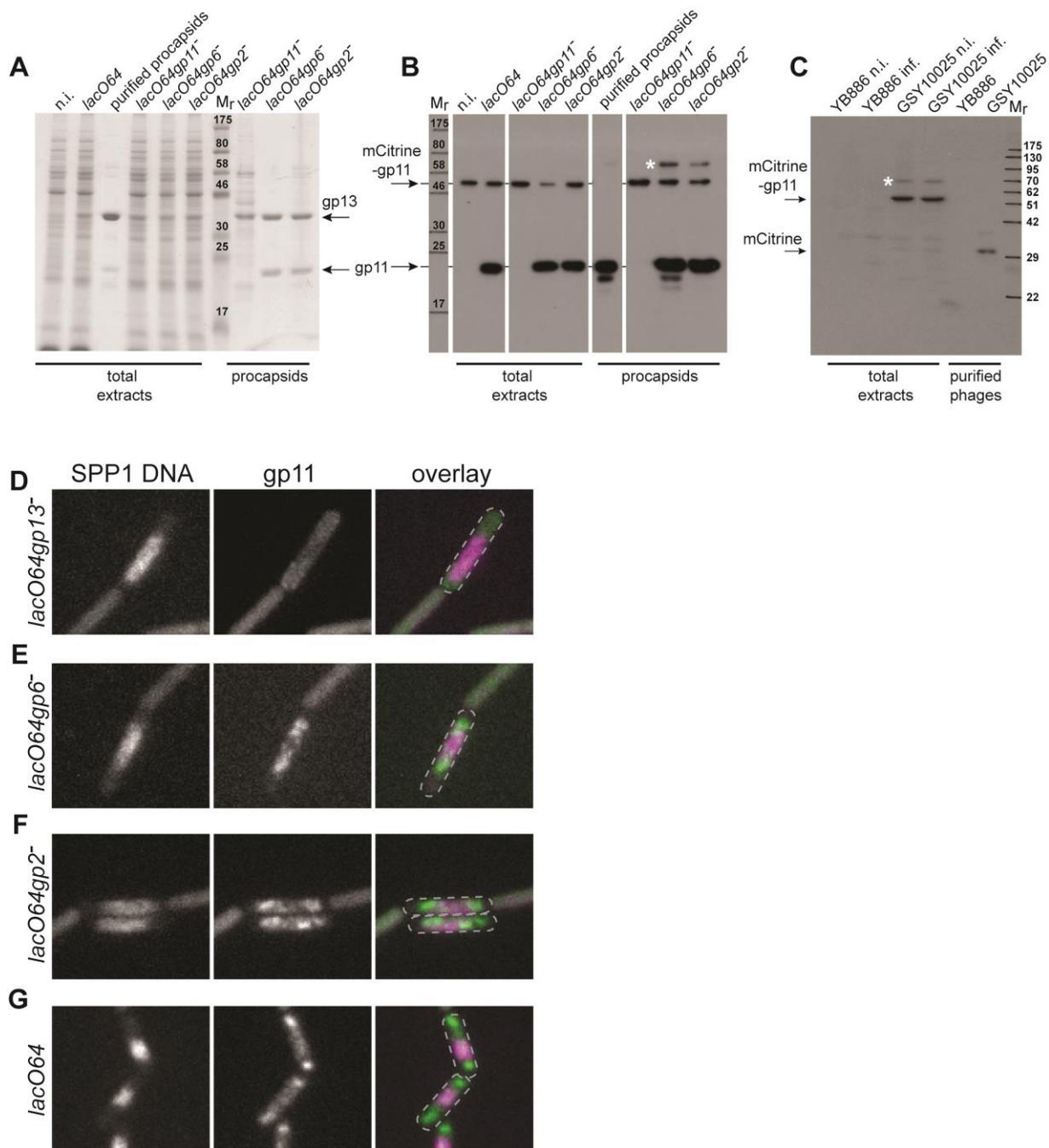
(A) Number of intracellular infectious particles in *B. subtilis* GSY10004 (producing LacI-mCherry) cells infected with SPP1 wild-type (black), SPP1 *delX110* (blue), and SPP1 *delX110lacO64* (also abbreviated SPP1/*lacO64* in this work) (red). Infectious particles were scored by titration after lysis of bacteria with 10% chloroform at different time points p.i. and divided by the number of bacteria infected at an OD<sub>600nm</sub> of 0.8 (10<sup>8</sup> cfu/mL). The results are the average of three independent experiments. (B) Quantification of total DNA (empty circles) and DNase-protected DNA (filled circles) by Qubit in cell extracts from *B. subtilis* GSY10004 non-infected (grey) and infected with SPP1/*lacO64* (red) prepared at different time points p.i.. Non-infected and infected bacteria originated from the same culture split prior to infection. Diamonds connected with eye guiding lines display the culture optical density at 600nm. The results are the average of three independent experiments. (C) Localization of TetR-mCherry (red in the overlay) binding to an array of *tetO* repeats, inserted between the *soj* and *spo0J* loci in a region proximal to the *B. subtilis* origin of replication, during exponential growth in LB medium at 37°C. The cells membrane contour is stained with TMA-DPH (blue in the overlay). The scale bar represents 2 μm. (D) Distribution of TetR-mCherry foci per bacterium in the reporter system of (C). Data are the average of counts from three independent experiments. Around 200 bacteria were scored in each experiment. The average number of TetR-mCherry foci per bacterium is 2.7. (E) Localization of *B. subtilis* replication origin region (red in the overlay) and of SPP1 DNA (blue in the overlay) using the reporter system of (C) combined with a LacI-CFP fusion protein in *B. subtilis* GSY10051 cells infected with SPP1/*lacO64gp2*<sup>-</sup>. Grey dotted lines display cell outlines defined from phase imaging. (F) Change in *B. subtilis* GSY10004 bacterial genome copy number during infection with SPP1/*lacO64* determined by qPCR of bacterial gene *gyrA*. (G) SPP1 genome copy number during the infection in (F) determined by qPCR of SPP1 gene 6.



**Fig. S2**

**Fig. S2. Imaging of the SPP1 DNA compartment and of *B. subtilis* replisome proteins during SPP1 infection.**

(A) Determination of the multiplicity of infection (MOI) in a population of SPP1-infected bacteria following a Poisson distribution and computed from the number of non-infected bacteria (segmented in green on the left) (see *SI Appendix, Materials and Methods*). The experimental counts of cells with one (segmented in magenta) and two (blue) SPP1 DNA foci fit to the theoretical distribution of mono-infected and bi-infected bacteria, respectively (right). (B) Experimental setup to image SPP1-infected cells in agarose pads illustrated by visualization of the DNA compartment in *B. subtilis* GSY10004 mono-infected with SPP1/*acO64* at early and late times p.i.. SPP1 DNA is in magenta and the cells membrane contour is stained with TMA-DPH displayed in green. (C) Localization of host replisome proteins in non-infected (left) and in SPP1/*acO64*-infected (right) bacteria late p.i. (see (B)). All strains produce constitutively LacI-mCherry to label phage DNA and one GFP (DnaE, DnaN, SsbA, DnaB and DnaC) or mCFP (DnaG) fusion to label a bacterial replisome protein. The display is as in Fig. 2A. The scale bar represents 2  $\mu\text{m}$ . Arrowheads show the position of host proteins foci in non-infected bacteria and arrows identify foci of SsbA not-colocalizing with SPP1 DNA in infected bacteria. Quantification of replisome proteins foci co-localization with SPP1 DNA is presented in Fig. 2B while function and features of each protein are compiled in Table S2.



**Fig. S3. Presence of gp11 and mCitrine-gp11 in phage structures.**

(A-C) Analysis of extracts from *B. subtilis* GSY10025 cells infected with different SPP1 phage mutants and of viral structures purified from infected GSY10025 cells. Phage mutants used for infection are labeled above the gel lanes. Lysates of non-infected bacteria (n.i.) and purified procapsids competent for DNA packaging *in vitro* are controls. (A) Coomassie Blue-stained gel. Bands of the major capsid protein gp13 and of the scaffolding protein gp11 are labeled. Mr, molecular weight marker. (B) Anti-gp11 western

blot detecting gp11 and mCitrine-gp11. Note that particles sedimenting at the position of procapsids from lysates of SPP1*lacO64gp11*<sup>-</sup> infection of strain GSY10025 contain only a small amount of mCitrine-gp11 and no gp11, resulting on gp13 polymorphic structures that spread through the glycerol gradient used for particles separation (see *SI Appendix, Materials and Methods*). The white asterisk indicates a band of unknown origin recognized by anti-gp11 polyclonal antibodies. (C) Anti-GFP western blot to detect presence of mCitrine in extracts of *B. subtilis* strains producing mCitrine-gp11 (GSY10025) or not (YB886) infected with SPP1*lacO64* (left) and in phage particles purified from both cell types (right). The white asterisk indicates a band of unknown origin recognized by anti-GFP antibodies. (D-G) Localization of SPP1 DNA (magenta in overlay) and of mCitrine-gp11 (green in overlay) in *B. subtilis* GSY10025 infected with SPP1*lacO64gp13*<sup>-</sup> (defective in procapsid assembly) (D), SPP1*lacO64gp6*<sup>-</sup> (defective in portal protein production; leads to assembly of procapsids without portal that do not package DNA) (E), SPP1*lacO64gp2*<sup>-</sup> (defective in DNA packaging) (F) and SPP1*lacO64* (G). Images were taken at late stages of infection. Grey dotted lines display cell outlines defined from brightfield imaging. The scale bar represents 2 μm.

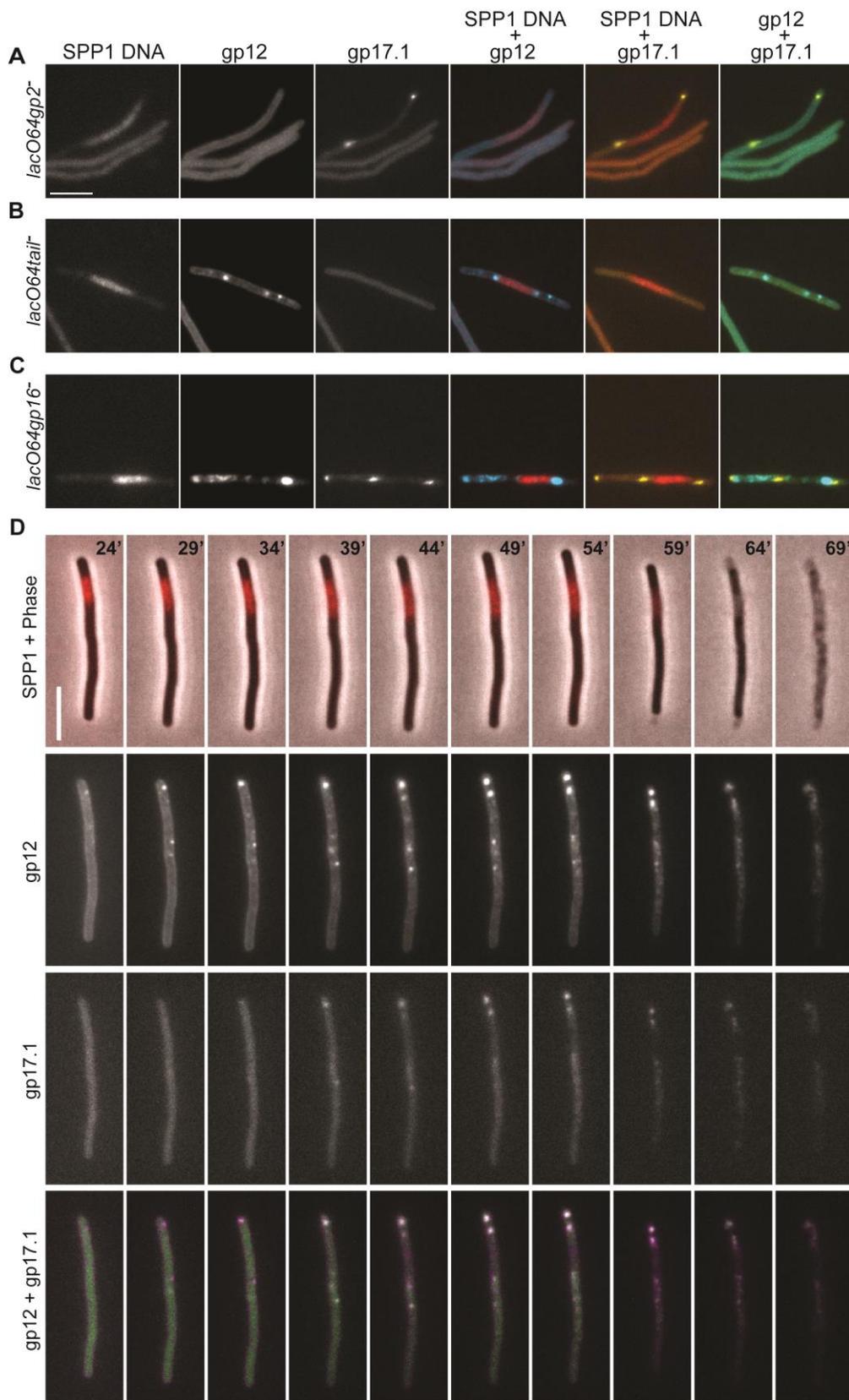
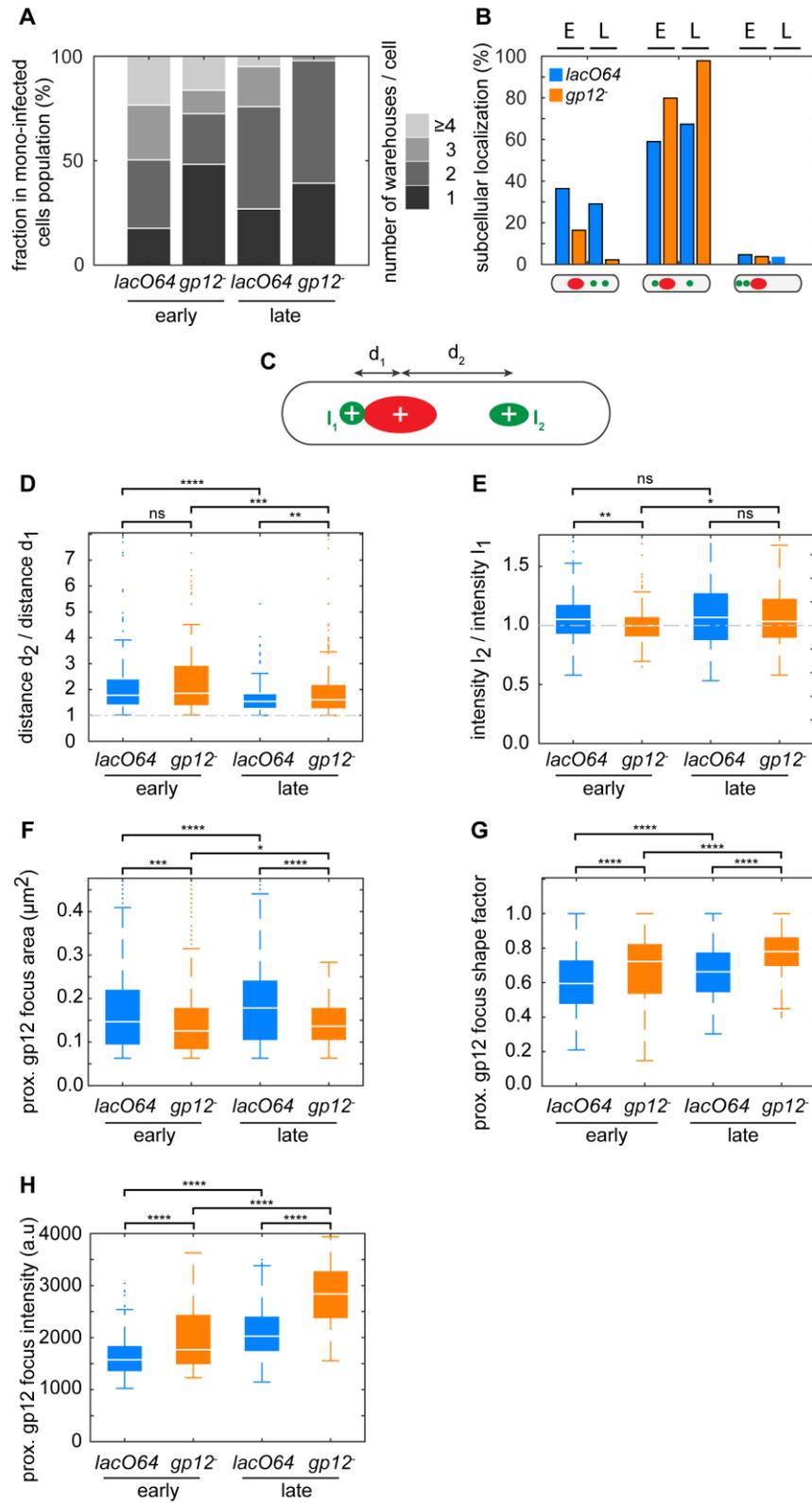


Fig. S4

**Fig. S4. Localization of the SPP1 DNA compartment, capsids and tails in infected bacteria.**

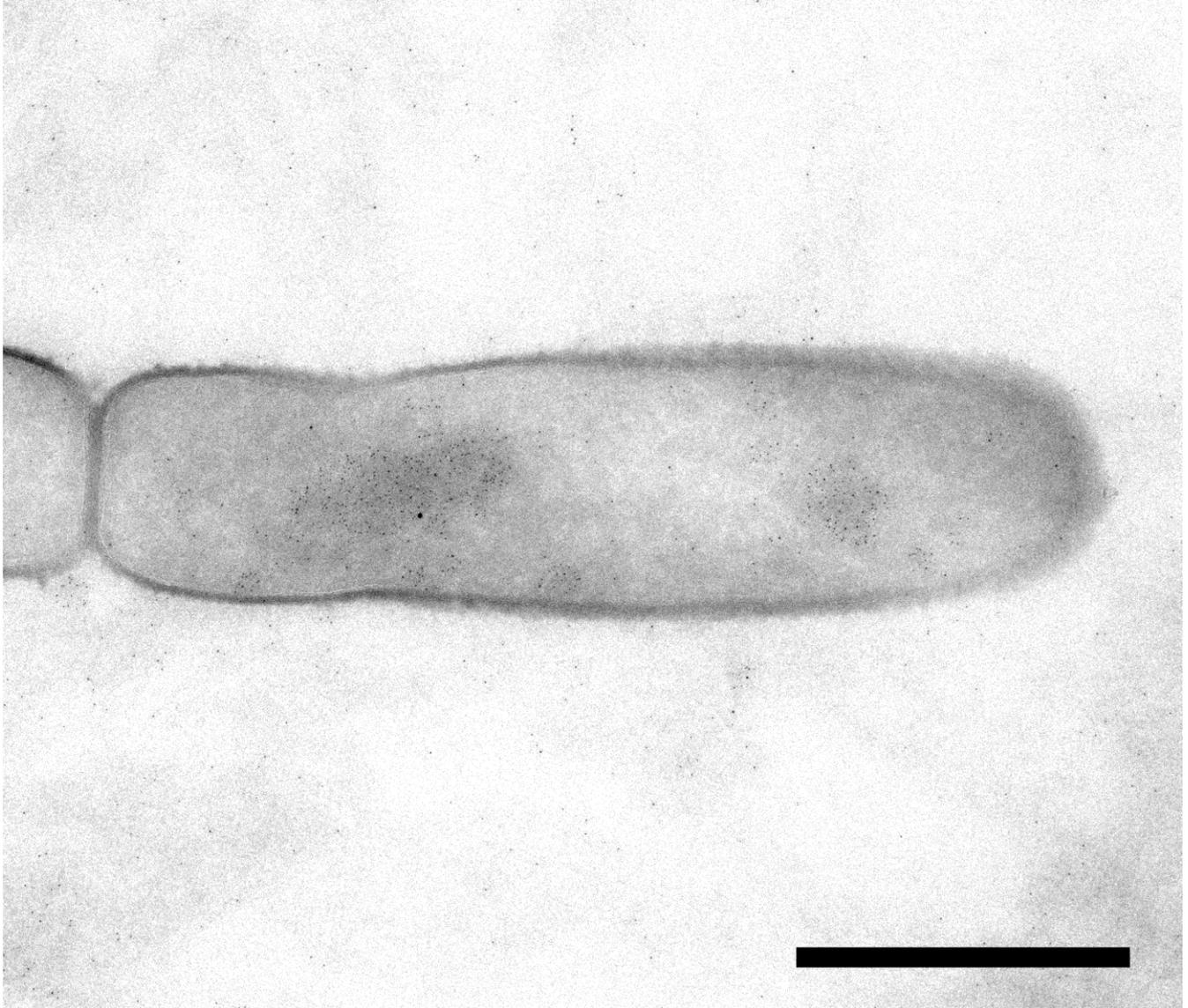
(A-C) Infection of *B. subtilis* GSY10096 filamented cells with SPP1/*lacO64gp2<sup>-</sup>* (defective in DNA packaging) (A), SPP1/*lacO64tail<sup>-</sup>* (defective in tail assembly) (B), and SPP1/*lacO64gp16<sup>-</sup>* (defective in formation of the capsid connector interface for phage tail binding) (C). The fluorescent reporters of SPP1 DNA, capsids, and tails were LacI-mCherry (red in overlays), gp12-mCFP (blue) and gp17.1-mCitrine (yellow), respectively. Images were taken late p.i. (SI Appendix Fig. S2B). (D) Time-lapse of *B. subtilis* GSY10096 infection with SPP1/*lacO64*. SPP1 DNA imaging (red) and phase contrast are overlaid on the figure top row to show the phage DNA compartment and its loss of organization at the onset of cell lysis. Time p.i. is displayed on the top right corner of each image in the top row. Gp12-mCFP (displayed in magenta in the bottom row overlay) and gp17.1-mCitrine (green in the overlay) fluorescence signals are presented in the rows underneath. The scale bars represent 5  $\mu$ m.



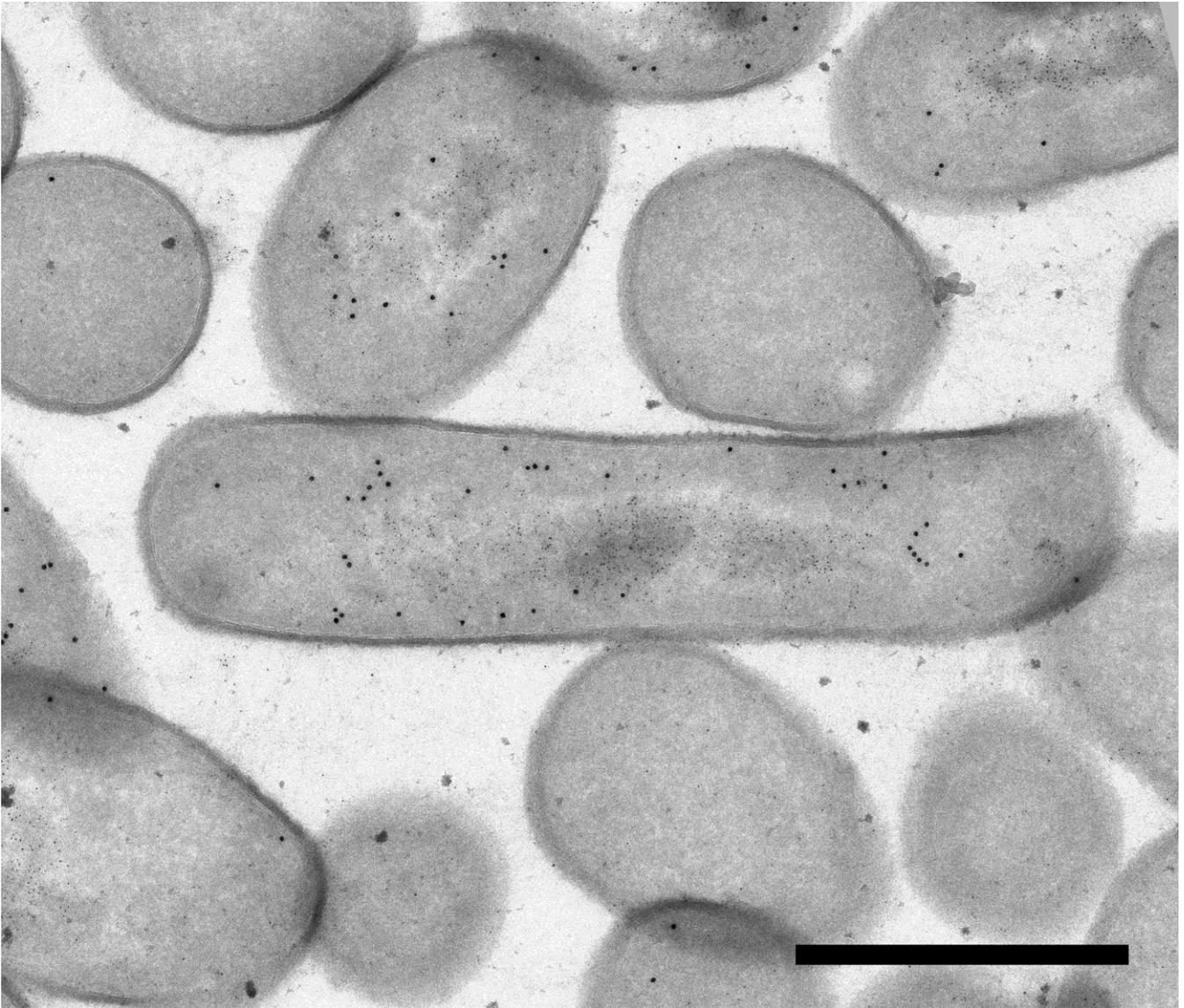
**Fig. S5**

**Fig. S5. Spatial organization of the SPP1 DNA compartment and of warehouses in the cell.**

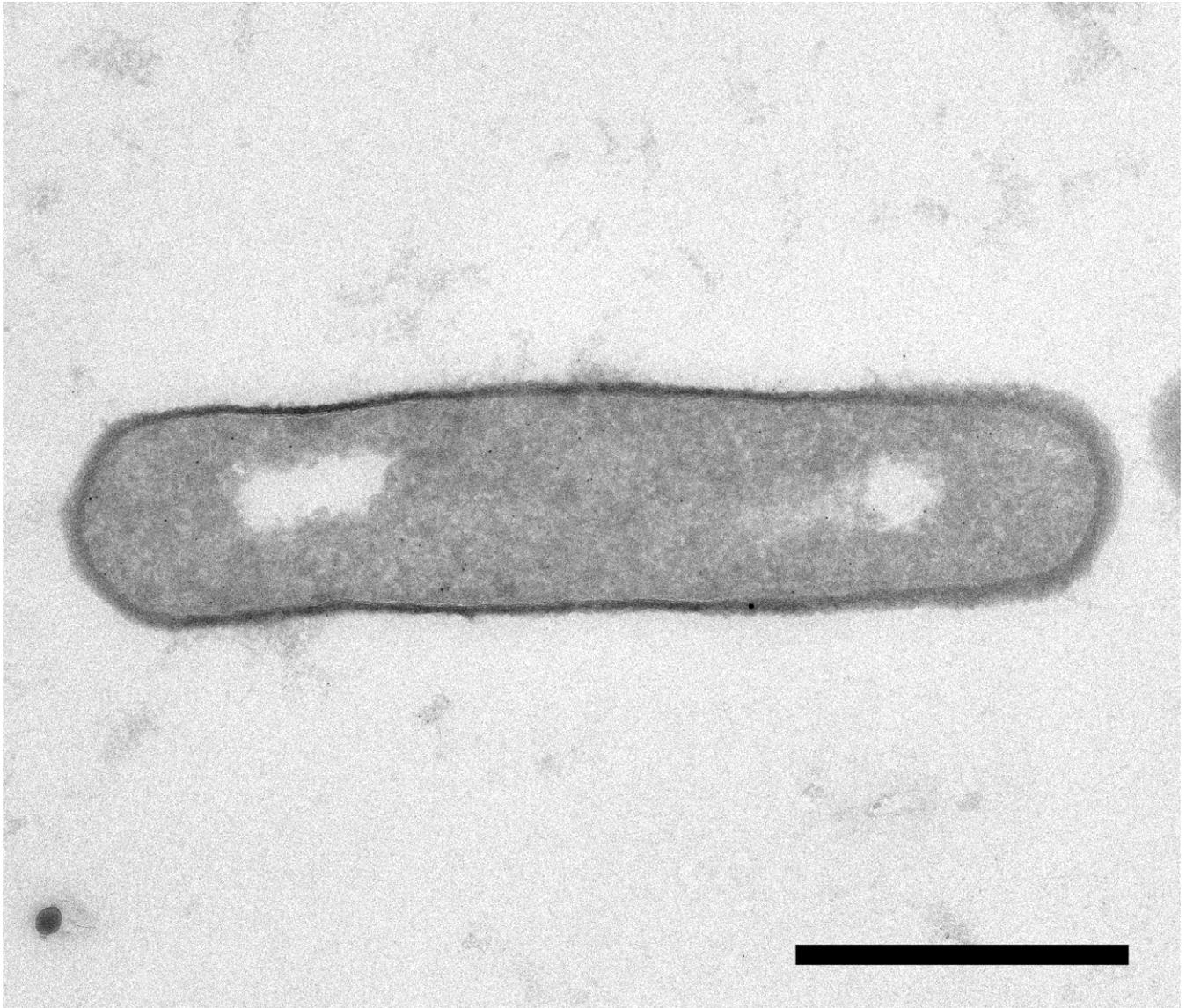
(A) Distribution of the number of warehouses (foci of gp12-mCitrine-containing particles) in GSY10024 mono-infected cells (one SPP1 DNA focus) with SPP1 *lacO64* (abbreviated *lacO64* in the figure) or with SPP1 *lacO64gp12* (abbreviated *gp12* in the figure) at early and late times p.i.. Analysis was carried out with Fig. 6J datasets, including more than 500 cells from three independent infections. (B) Localization of warehouses relative to the SPP1 DNA compartment position in the subset of cells in (A) with two warehouses at early (E) and late (L) times p.i., according to the topology depicted underneath the histogram bars. More than 130 cells from three independent infections were analysed. Red, DNA compartment; green, warehouse. (C) Scheme illustrating measurements of distance and fluorescence intensity parameters in mono-infected bacteria whose viral DNA compartment is flanked by one warehouse on each side, the most frequent topology found in cells with two warehouses (B). “+” is the center of mass of the fluorescent focus. (D) Ratio between distances of the two warehouse centers of mass to the SPP1 DNA compartment center of mass ( $d_2/d_1$ ; see (C)) in the sub-set of cells from (B). (E) Ratio of fluorescence average intensity between the warehouses from cells in (B) ( $I_2/I_1$ ; see (C)). More than 130 cells from three independent infections were analyzed in (B,D and E). (F-H) Average area (F), shape factor (minimal/maximal diameters) (G) and average fluorescence intensity (H) of the warehouse proximal to the DNA compartment in all cells from (A). More than 500 cells from three independent infections were analyzed. In panels (D-H), the boxplot shows median of the sample data as a white solid line and draws point as outliers if they are greater than  $q_3 + w(q_3 - q_1)$  or less than  $q_1 - w(q_3 - q_1)$ , where  $q_1$  and  $q_3$  are the 25<sup>th</sup> and 75<sup>th</sup> percentiles of the sample data, respectively and  $w = 1.5$ . Statistical significance between conditions was calculated using the Mann–Whitney non-parametric test (\*\*\*\* =  $P < 0.0001$ ; \*\*\* =  $0.0001 < P < 0.001$ ; \*\* =  $0.001 < P < 0.01$ ; \* =  $0.01 < P < 0.05$ ; ns =  $P > 0.05$ ). a.u., arbitrary units.



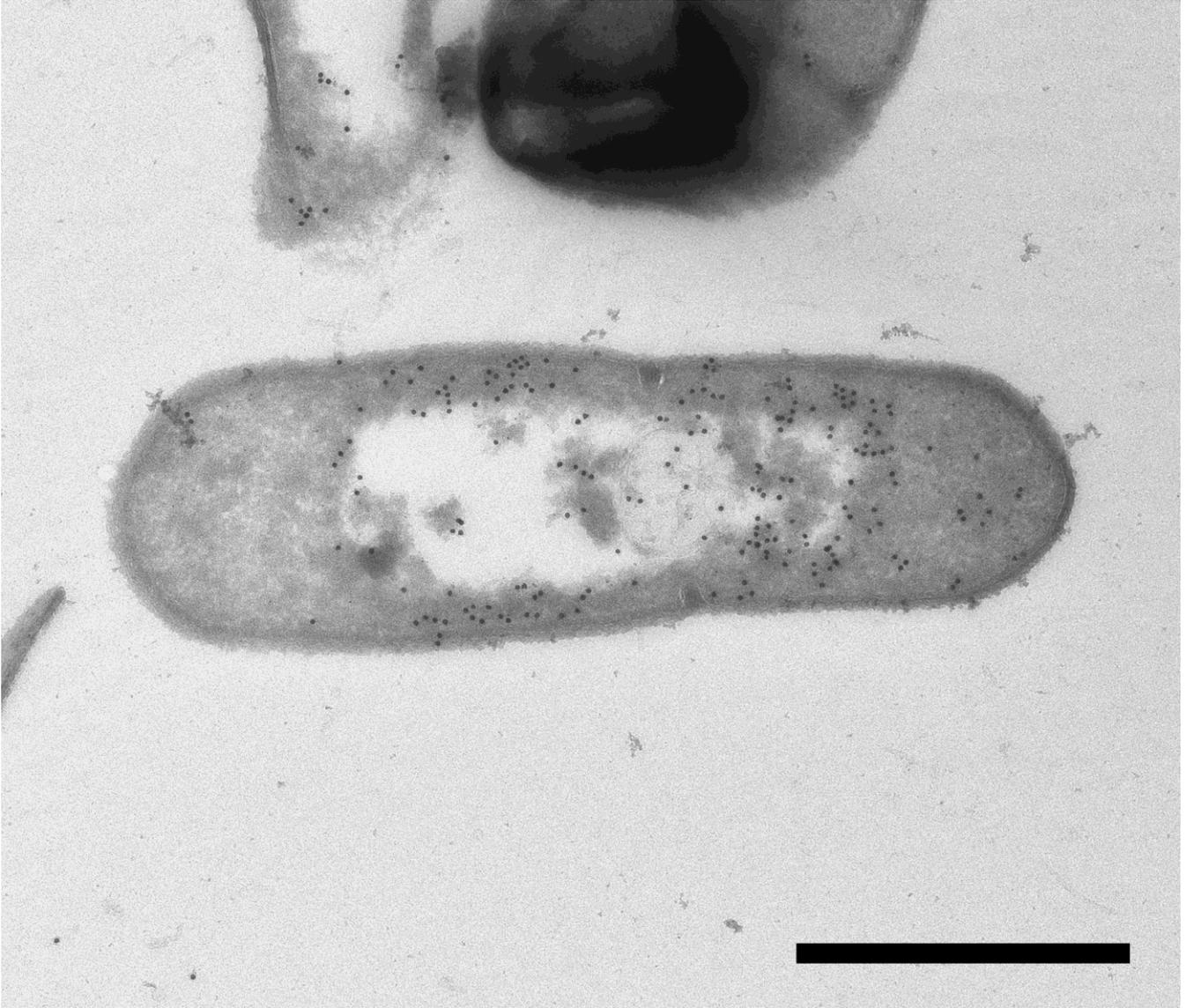
**High resolution Fig. 5A**



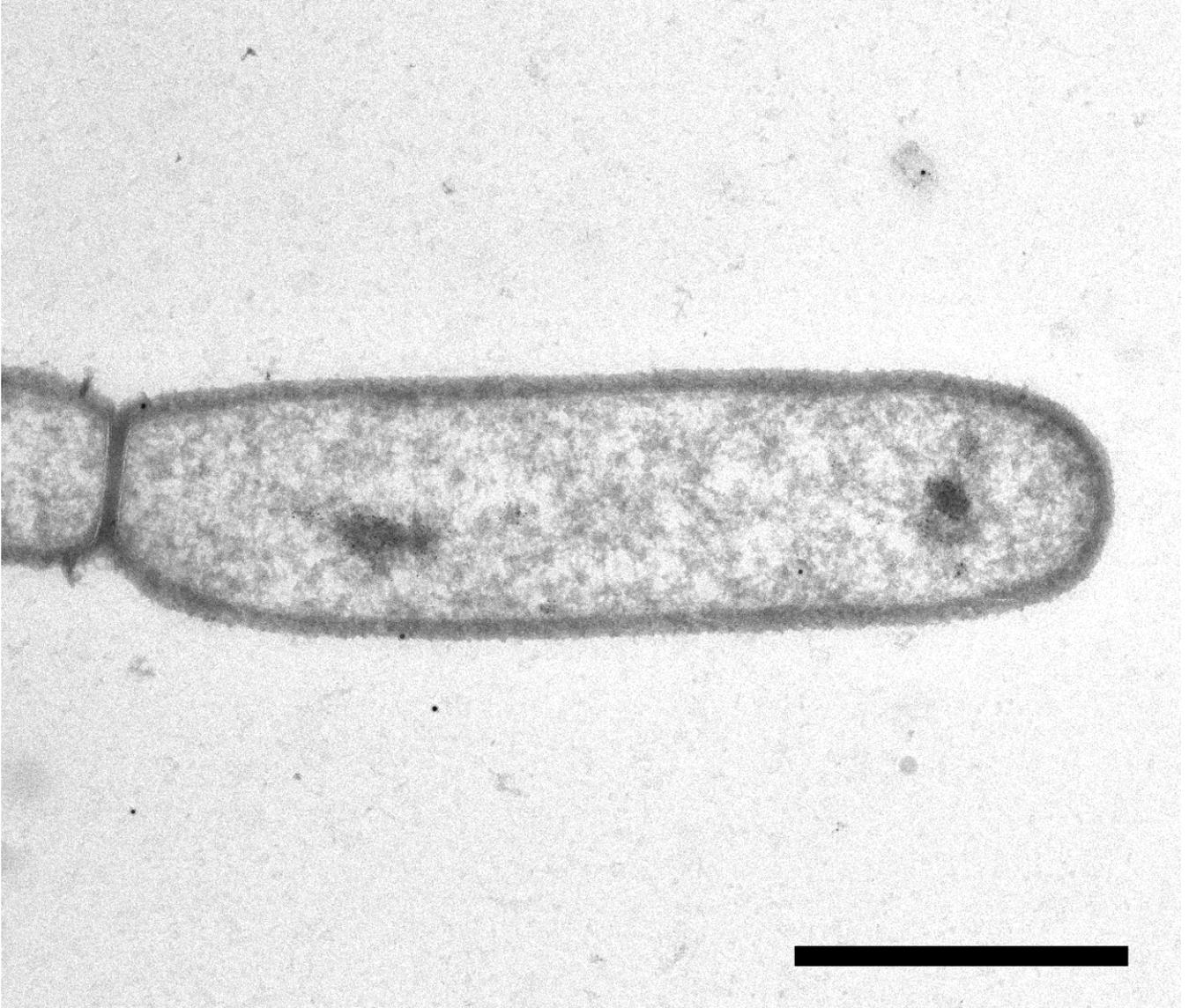
**High resolution Fig. 5B**



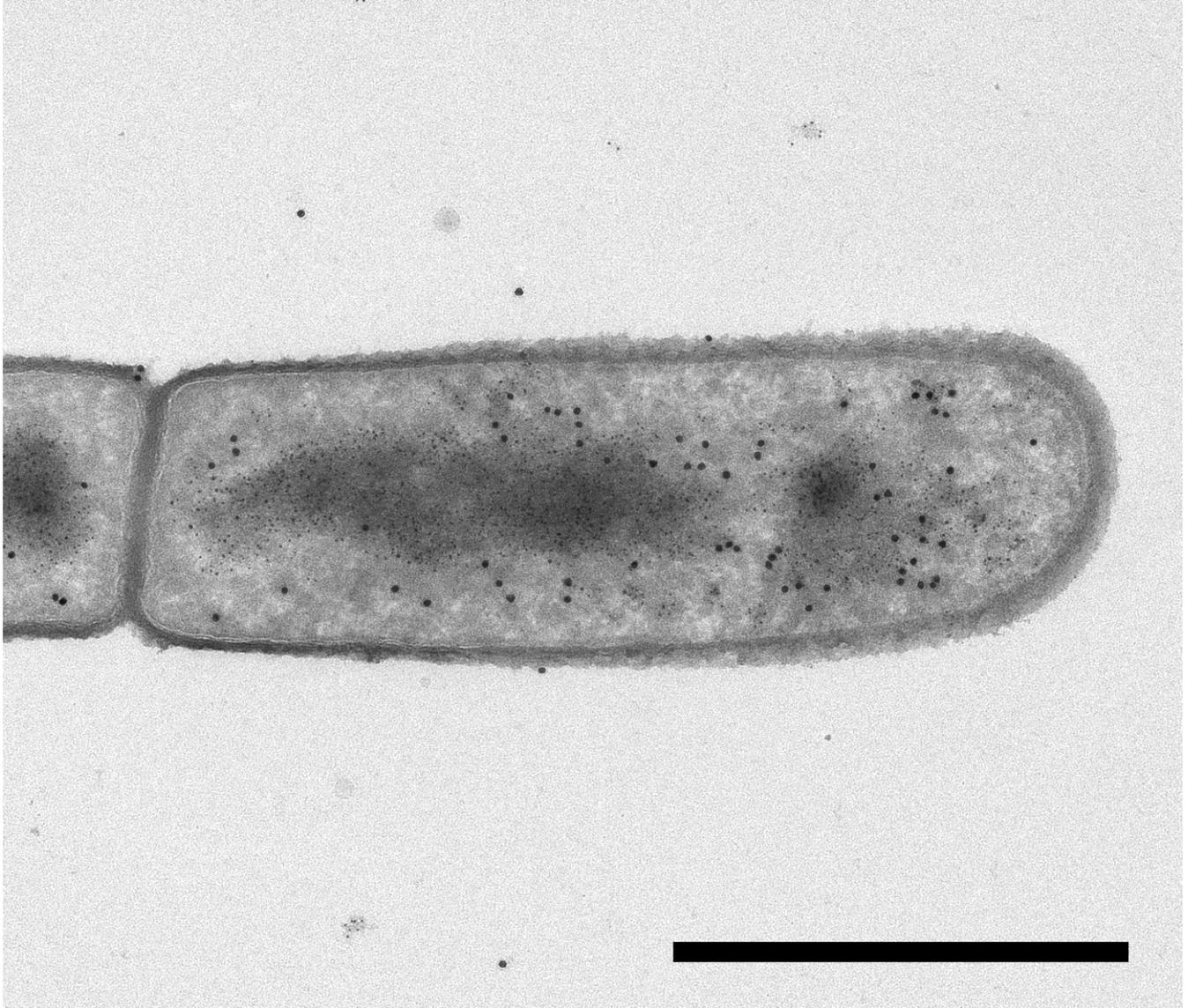
**High resolution Fig. 5C**



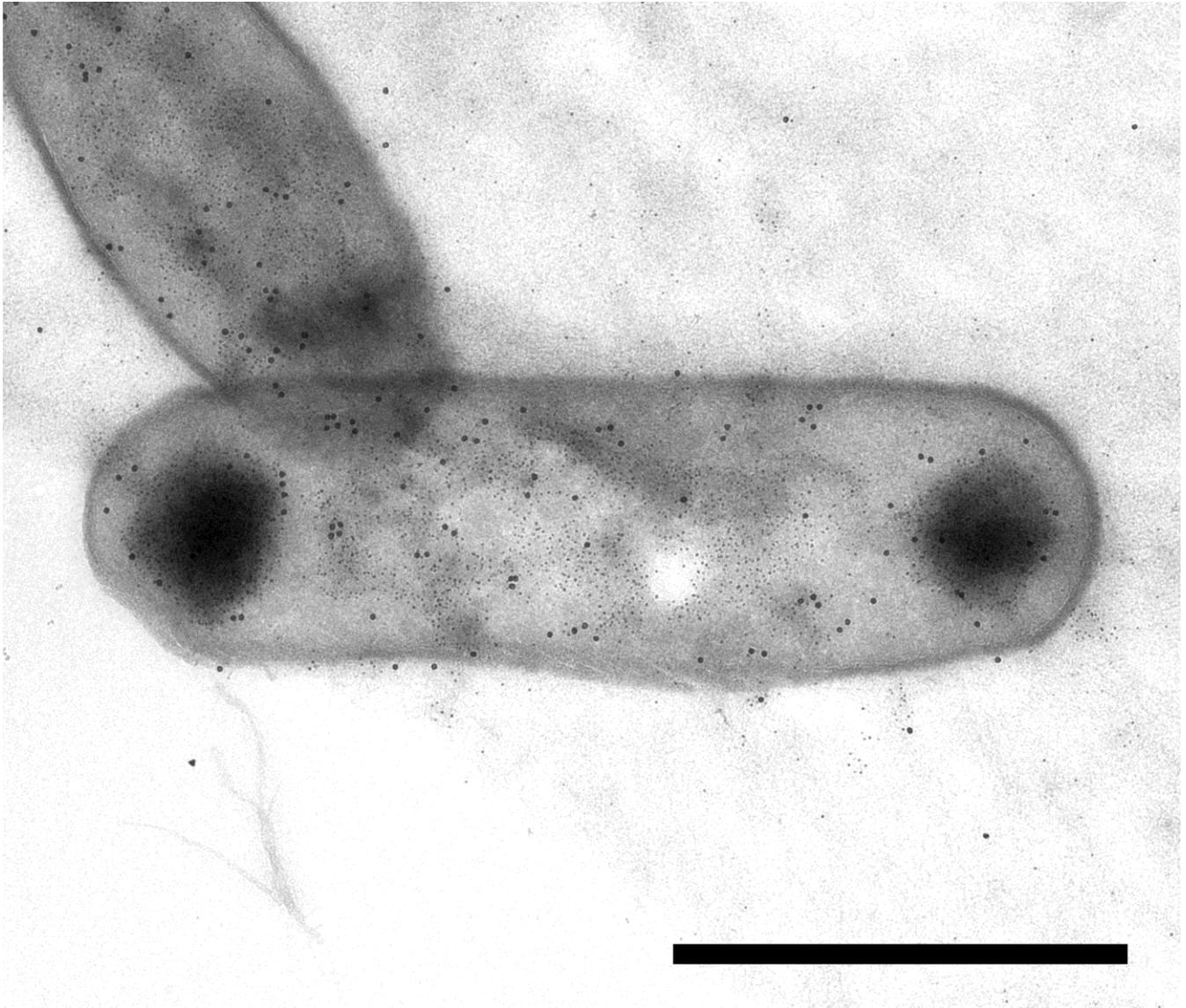
High resolution Fig. 5D



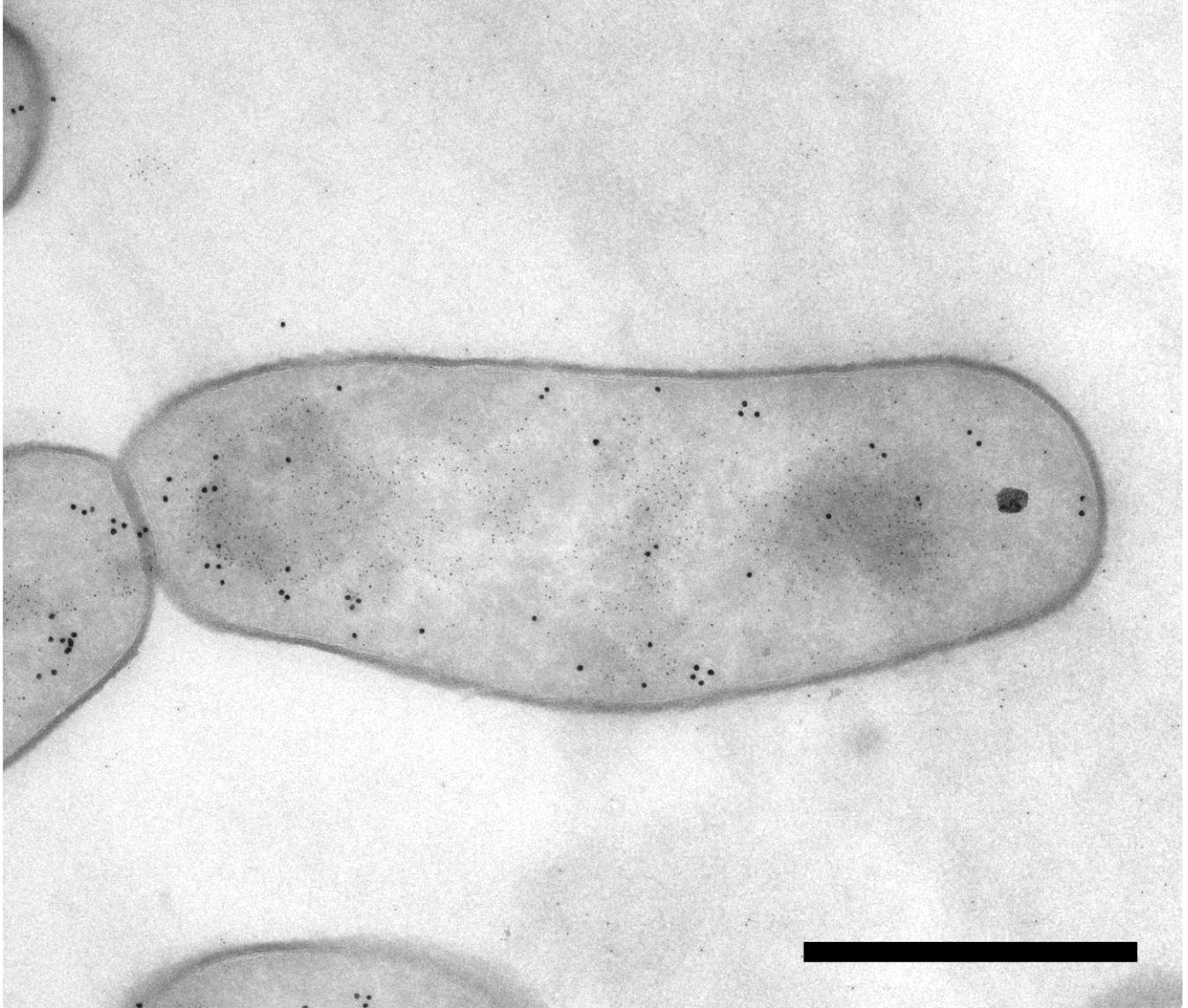
High resolution Fig. 5E



High resolution Fig. 5F



High resolution Fig. 6E



**High resolution Fig. 6G**

**Table S1: SPP1 proteins studied in this work involved in phage DNA replication and viral particle assembly.**

Their function (14), role on SPP1 multiplication (14), and type of FP fusion used in this work (Figs. 3,5 and 6 and *SI Appendix* Figs. S3 and S4) are presented.

<b>SPP1 protein</b>	<b>Function</b>	<b>Essential for SPP1</b>	<b>FP fusion</b>
<b>Viral particle assembly</b>			
gp1	SPP1 TerS; phage DNA recognition and packaging	yes	NA
gp2	SPP1 TerL; phage DNA packaging	yes	NA
gp6	procapsid and capsid portal protein, DNA packaging	yes	NA
gp7	procapsid and capsid minor protein ; DNA transfer to host cell	no	NA
gp11	internal procapsid scaffolding protein	yes	N-Ter
gp12	DNA-filled capsid auxiliary protein	no	C-Ter
gp13	major capsid protein	yes	NA
gp15	capsid connector adaptor protein	yes	NA
gp16	capsid connector stopper protein	yes	NA
gp17.1	major tail tube protein	yes	C-Ter
<b>DNA replication</b>			
gp34.1	5'-3' exonuclease ; switch theta to sigma mode of replication	no	C-Ter
gp35	recT-like recombinase	yes	N and C-Ter
gp38	SPP1 replication origin binding binding protein and replication re-start	yes	NA
gp39	gp40 helicase loader	yes	NA
gp40	replicative DNA helicase	yes	C-Ter

**Table S2. *B. subtilis* replisome proteins.**

Function, essential role in SPP1 replication (15), localization in non-infected ((16) and this work) and in infected bacteria (this work), and type of FP fusion used in this work (Figs. 2,3 and *SI Appendix* Fig. S2).

<b><i>B. subtilis</i> protein</b>	<b>function</b>	<b>essential for SPP1</b>	<b>localization in non-infected bacterium</b>	<b>localization change to SPP1 DNA</b>	<b>FP fusion</b>
<b>PoIC</b>	DNA polymerase	yes	mid cell	yes	C-Ter
<b>DnaE</b>	DNA polymerase	yes	mid cell	yes	C-Ter
<b>DnaX</b>	DNA pol III $\tau$ subunit, assembly of the replisome, clamp loader	yes	mid cell	yes	N-Ter
<b>DnaN</b>	DNA pol III $\beta$ subunit, sliding clamp	yes	mid cell	yes	N-Ter
<b>DnaG</b>	primase	yes	homogeneous	yes	N-Ter
<b>SsbA</b>	single-stranded DNA-binding protein	no	mid cell	yes	N-Ter
<b>DnaB</b>	initiation of DNA replication, primosome component	no	mid cell	yes	N-Ter
<b>DnaC</b>	replicative helicase	no	homogeneous	no	N-Ter

**Table S3. Bacterial strains, phages, plasmids, antibodies and primers used in this study.**

<b>Bacterial strains</b>		
<i>E. coli</i> F- $\Phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ ( <i>lacZYA-argF</i> ) U169 <i>recA1 endA1 hsdR17</i> ( <i>rk</i> , <i>mk</i> <sup>+</sup> ) <i>phoA supE44 <math>\lambda</math>-thi 1 gyrA96 relA1</i>	(17)	DH5 $\alpha$
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math></i>	(18)	YB886
<i>B. subtilis</i> [ <i>his met</i> ] <i>sup- leu trpC2</i>	(19)	HA101B
<i>B. subtilis</i> [ <i>metB5</i> ] <i>sup-3 amyE trpC2 xin-1 attSP<math>\beta</math></i>	(20)	BG295
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>cfp mls</i> )	(4)	GSY10000
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> )	This work	GSY10004
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> -12- <i>mcitrine kan</i> )	This work	GSY10024
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> - <i>mcitrine-11 kan</i> )	This work	GSY10025
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> -34.1- <i>mcitrine kan</i> )	This work	BG1837
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> - <i>mcitrine-35 kan</i> )	This work	BG1831
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> -35- <i>mcitrine kan</i> )	This work	BG1833
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> -40- <i>mcitrine kan</i> )	This work	BG1829
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> - <i>mcfp-dnaG spc</i> )	This work	GSY10031
<i>B. subtilis amyE trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>sacA::</i> (P <sub>spac</sub> - <i>mcfp-dnaX spc</i> )	This work	GSY10060
<i>B. subtilis</i> JJS59 <i>amyE::</i> (P <sub>xyI</sub> - <i>polC-gfp spc</i> )	(16)	PolC-GFP
<i>B. subtilis trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>amyE::</i> (P <sub>xyI</sub> - <i>polC-gfp spc</i> )	This work	GSY10006
<i>B. subtilis</i> JJS59 <i>amyE::</i> (P <sub>xyI</sub> - <i>gfp-dnaN spc</i> )	(16)	GFP-DnaN
<i>B. subtilis trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>amyE::</i> (P <sub>xyI</sub> - <i>gfp-dnaN spc</i> )	This work	GSY10008
<i>B. subtilis</i> JJS59 <i>amyE::</i> (P <sub>xyI</sub> - <i>gfp-ssbA spc</i> )	(16)	GFP-SsbA
<i>B. subtilis trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>amyE::</i> (P <sub>xyI</sub> - <i>gfp-ssbA spc</i> )	This work	GSY10010
<i>B. subtilis</i> JJS59 <i>amyE::</i> (P <sub>xyI</sub> - <i>gfp-dnaB spc</i> )	(16)	GFP-DnaB
<i>B. subtilis trpC2 metB5 xin-1 attSP<math>\beta</math> thrC::</i> (P <sub>pen</sub> - <i>lacI</i> $\Delta$ 11- <i>mcherry mls</i> ) <i>amyE::</i> (P <sub>xyI</sub> - <i>gfp-dnaB spc</i> )	This work	GSY10011
<i>B. subtilis</i> JJS59 <i>amyE::</i> (P <sub>xyI</sub> - <i>dnaE-gfp spc</i> )	(16)	DnaE-GFP

<i>B. subtilis trpC2 metB5 xin-1 attSPβ thrC::(P<sub>pen</sub>-lacIΔ11-mcherry mls) amyE::(P<sub>xyI</sub>-dnaE-gfp spc)</i>	This work	GSY10012
<i>B. subtilis</i> JJS59 <i>amyE::(P<sub>xyI</sub>-gfp-dnaC spc)</i>	(16)	GFP-DnaC
<i>B. subtilis trpC2 metB5 xin-1 attSPβ thrC::(P<sub>pen</sub>-lacIΔ11-mcherry mls) amyE::(P<sub>xyI</sub>-gfp-dnaC spc)</i>	This work	GSY10014
<i>B. subtilis trpC2 metB5 xin-1 attSPβ thrC::(P<sub>pen</sub>-lacIΔ11-mcherry mls) sacA::(P<sub>spac</sub>-40-mcitrine kan) amyE::(P<sub>xyI</sub>-mcfp-dnaX spc)</i>	This work	GSY10061
<i>B. subtilis trpC2 metB5 xin-1 attSPβ thrC::(P<sub>pen</sub>-lacIΔ11-mcherry mls) sacA::(P<sub>spac</sub>-17.1-mcitrine kan)</i>	This work	GSY10095
<i>B. subtilis trpC2 metB5 xin-1 attSPβ thrC::(P<sub>pen</sub>-lacIΔ11-mcherry mls) sacA::(P<sub>spac</sub>-17.1-mcitrine kan) amyE::(P<sub>xyI</sub>-gp12-mcfp spc)</i>	This work	GSY10096
<i>B. subtilis trpC2 spo0J::(tetO167 neo) amyE::(P<sub>xyI</sub>-tetR-mcherry spc)</i>	Kindly provided by Heath Murray BGSC 1A1087	HM1049
<i>B. subtilis trpC2 metB5 xin-1 attSPβ trpC2 thrC::(P<sub>pen</sub>-lacIΔ11-cfp mls) amyE::(P<sub>xyI</sub>-tetR-mcherry spc)</i>	This work	GSY10050
<i>B. subtilis trpC2 metB5 xin-1 attSPβ trpC2 thrC::(P<sub>pen</sub>-lacIΔ11-cfp mls) spo0J::(tetO167 neo) amyE::(P<sub>xyI</sub>-tetR-mcherry spc)</i>	This work	GSY10051

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## Phages

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SPP1 <sup>wt</sup> : SPP1 wild-type lytic siphophage	(21)	<i>wt</i>
SPP1 <sup>delX110</sup> : SPP1 <sup>wt</sup> derivative carrying deletion <i>delX110</i>	(22)	<i>delX110</i>
SPP1 <sup>delX110lacO64</sup> : SPP1 <sup>delX110</sup> derivative carrying an array of <i>lacO</i> operators	(4)	<i>lacO64</i>
SPP1 <sup>sus34.1</sup> : phage SPP1 defective in gene <i>34.1</i>	(23)	<i>gp34.1</i> <sup>-</sup>
SPP1 <sup>delX110lacO64sus34.1</sup> : SPP1 <sup>sus34.1</sup> derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp34.1</i> <sup>-</sup>
SPP1 <sup>sus12</sup> : phage SPP1 defective in gene <i>40</i>	(15)	<i>gp40</i> <sup>-</sup>
SPP1 <sup>delX110lacO64sus12</sup> : SPP1 <sup>sus12</sup> derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp40</i> <sup>-</sup>
SPP1 <sup>sus31</sup> : phage SPP1 defective in gene <i>13</i>	(8)	<i>gp13</i> <sup>-</sup>
SPP1 <sup>delX110lacO64sus31</sup> : SPP1 <sup>sus31</sup> derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp13</i> <sup>-</sup>
SPP1 <sup>sus19</sup> : phage SPP1 defective in gene <i>2</i>	(6)	<i>gp2</i> <sup>-</sup>
SPP1 <sup>delX110lacO64sus19</sup> : SPP1 <sup>sus19</sup> derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp2</i> <sup>-</sup>
SPP1 <sup>del12</sup> : phage SPP1 with a deletion in gene <i>12</i>	(24)	<i>gp12</i> <sup>-</sup>
SPP1 <sup>delX110lacO64del12</sup> : SPP1 <sup>del12</sup> derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp12</i> <sup>-</sup>
SPP1 <sup>sus115</sup> : phage SPP1 defective in gene <i>6</i>	(22)	<i>gp6</i> <sup>-</sup>

SPP1 <del>X110</del> lacO64sus115: SPP1sus115 derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp6</i>
SPP1sus7: phage SPP1 defective in gene 11	(8)	<i>gp11</i>
SPP1 <del>X110</del> lacO64sus7: SPP1sus7 derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp11</i>
SPP1sus117: phage SPP1 defective in gene 16	(5, 25)	<i>gp16</i>
SPP1 <del>X110</del> lacO64sus117: SPP1sus117 derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64gp16</i>
SPP1sus9: phage SPP1 defective in tail assembly gene	(8, 26)	<i>tail</i>
SPP1 <del>X110</del> lacO64sus9: SPP1sus9 derivative carrying an array of <i>lacO</i> operators	This work	<i>lacO64tail</i>

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### Plasmids

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pHP13	(27)	NA
pJ1 ( <i>kan:mcherry</i> )	Kindly provided by A.O. Henriques	NA
pKL190	(28)	NA
pDH88	(29)	NA
pJexpress404 ( <i>bla:mcitrine</i> )	This work (DNA2:0)	NA
pDR200 ( <i>bla:mcfp</i> )	Kindly provided by T. Doan and D. Rudner	NA
pSacKan <i>bla sacA::(kan)</i>	Kindly provided by D. Zeigler from BGSC	NA
pSG1190 <i>bla amyE:(P<sub>xyI</sub>-mcfp spc)</i>	Kindly provided by D. Zeigler from BGSC	NA
pSG1192 <i>bla amyE:(P<sub>xyI</sub>-mcfp spc)</i>	Kindly provided by D. Zeigler from BGSC	NA
pAL1 P <sub>pen</sub> - <i>lacIΔ11-mcherry erm</i>	This work	NA
pPT300 <i>bla thrC::(P<sub>pen</sub>-lacIΔ11-mcherry erm)</i>	This work	NA
pAL19 <i>bla sacA::(P<sub>spac</sub> kan)</i>	This work	NA
pAL20 <i>bla sacA::(P<sub>spac</sub>-mcitrine kan)</i>	This work	NA
pAL21 <i>bla sacA::(P<sub>spac</sub>-mcitrine kan)</i>	This work	NA
pAL24 <i>bla sacA::(P<sub>spac</sub>-12-mcitrine kan)</i>	This work	NA
pAL25 <i>bla sacA::(P<sub>spac</sub>-mcitrine-11 kan)</i>	This work	NA
pML1837 <i>bla sacA::(P<sub>spac</sub>-34.1-mcitrine kan)</i>	This work	NA
pML1831 <i>bla sacA::(P<sub>spac</sub>-mcitrine-35 kan)</i>	This work	NA
pML1833 <i>bla sacA::(P<sub>spac</sub>-35-mcitrine kan)</i>	This work	NA
pML1829 <i>bla sacA::(P<sub>spac</sub>-40-mcitrine kan)</i>	This work	NA
pAL28 <i>bla amyE::(P<sub>xyI</sub>-mcfp spc)</i>	This work	NA

pAL29 <i>bla amyE::</i> (P <sub>xyI</sub> - <i>mcfp spc</i> )	This work	NA
pAL31 <i>bla amyE::</i> (P <sub>xyI</sub> - <i>mcfp-dnaG spc</i> )	This work	NA
pAL36 <i>bla amyE::</i> (P <sub>xyI</sub> - <i>mcfp-dnaX spc</i> )	This work	NA
pAL44 <i>bla amyE::</i> (P <sub>xyI</sub> -12- <i>mcfp spc</i> )	This work	NA
pAL53 <i>bla sacA::</i> (P <sub>spac-17.1</sub> - <i>mcitrine kan</i> )	This work	NA
pHM232 <i>bla amyE::</i> (P <sub>xyI</sub> - <i>tetR-mcherry spc</i> )	(30)	NA
pUC19-EJ1 vector with amplicons of SPP1, <i>B.s</i> and T7	This work	NA

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### Antibodies

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Anti-Rabbit IgG	Sigma Aldrich	Cat#A6667
Anti-SPP1 gp11 rabbit polyclonal serum	(8)	NA
Anti-SPP1 gp12 rabbit polyclonal serum	(24)	NA
Anti-SPP1 gp17.1 rabbit polyclonal serum	(31)	NA
Anti-SPP1 procapsids rabbit polyclonal serum	Kindly provided by S. Brasilès	NA
GFP Rabbit anti-Tag, polyclonal	Invitrogen	Cat#A6455
Goat Anti-Mouse IgG (H+L) Antibody, 5 nm Gold Conjugated	BBI Solutions	Cat#EM.GMHL5
Goat Anti-Rabbit IgG Antibody, 15 nm Gold Conjugated	BBI Solutions	Cat#EM.GAR15
Mouse Monoclonal against dsDNA	Abcam	Cat#ab27156

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### Primers

Primers	Sequence (5'-3')
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#### For cloning

Primer 1	CTGCTCGAGGGTTCCGGAATGGTCAGCAAGGGAGAGG
Primer 2	TTCGGATCCGCATGCTTATTTGTATAATTCGTCCATTCC
Primer 3	CGCGGATCCAGCCCGCCTAATGAGCGGGCTTTTTTTTACACAGCCCAG
Primer 4	GGCCGGATCCATTAATGATGATGATGATGATGACCAGAACCTTCTAA CAGCTGCATTAATGAATCG
Primer 5	CCGGAATCCAAAGGAGAAATCGATTAGACGCTCGAGCTCGAAGGCA GTGGAATGGTAAGCAAAGGCGAGGAACTCTTCACAGGCG
Primer 6	CGGGATCCTTATTATTTATACAGTTCATCCATACCATGAGTTATGCCA GCTGCCG
Primer 7	CCCCATCGATATGTCTAAGCGTATACCGGTTTCTTGC
Primer 8	CGCGGATCCTTACTCGAGAGTCGTTCCGCCGCCG
Primer 9	CCCCATCGATATGCCAGAAACGCCTATTATGGGACAAGATG
Primer 10	CGCGGATCCTTACTCGAGAGGAGCAGGAACAGTTCCCGGTG
Primer 11	TAGGATCGATAAAATGGCGAG

Primer 12	CTATACA <b>CTCGAG</b> TGCCTTTTGC
Primer 13	CCCC <b>ATCGAT</b> ATGGCAACTAAAAACAAGAGG
Primer 14	CCCC <b>CTCGAG</b> TTCAATTTGTTTCCCCTC
Primer 15	CCCC <b>ATCGAT</b> ATGAATCCAGAAGAAAAAACG
Primer 16	CCCC <b>CTCGAG</b> ACGATCATCAAACGAC
Primer 17	CCCC <b>ATCGAT</b> ATGAGTTTGAAAGAGCAGTTAGGTGAAGAGTTGTACG
Primer 18	CGC <b>GGATCC</b> TTATTACTCGAGCTGTGCTTCTGCTTGTAGCTTCT TCGC
Primer 19	CGC <b>GGATCC</b> CTATTCATTTGTTTCCC
Primer 20	CCG <b>AGGTACC</b> ATGGTTTCAAAGGCG
Primer 21	CG <b>AGGATCCC</b> CTTATAAAGTTCGTCCATGCC
Primer 22	CGC <b>GGATCC</b> ACTTAGAAGGATCTGGAATGGGAAATCGGATACC
Primer 23	CGAATTC <b>ATCGAT</b> TTATTTTAAAGATCGGTTCAATGTAACG
Primer 24	CGC <b>GGATCC</b> ACTTAGAAGGATCTGGAATGAGTTACCAAGCTTTATATCG
Primer 25	CCG <b>CTCGAG</b> TTAGTCTTTTATTTCAATTAATCC
Primer 26	CGA <b>GAATTC</b> ATGGTTTCAAAGGCG
Primer 27	CGA <b>ACTAGT</b> TTTATTACTTATAAAGTTCGTCCATGCC
Primer 28	CCG <b>GGTACC</b> ATGTCTAAGCGTATACCGGTTTCTTGC
Primer 29	CCG <b>CTCGAG</b> CGGTCCAGATCCTTCTAA AGTCGTTCCGCCGCCGCTTG

**For qPCR experiments**

SPP1-Fw	CGGGCTGAAATACCTGTGGA
SPP1-Rv	TAGCCCCTCCTCCGATTGTT
Bs-Fw	GAATACGGCAGAACGGCAA
Bs-Rv	TTCGTTTTGAAACCCCATGC

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**Table S4. Treatment and immunolabeling of thin sections of *B. subtilis* cells.**

Buffers, enzymatic treatment, antibodies labelling, and incubation conditions are presented.

	<b>no treatment</b>	<b>Benzonase treatment</b>	<b>trypsin treatment</b>	<b>incubation time</b>
<b>buffer A</b>	TBT	TBT + 25 U Benzonase	TBT + 10 $\mu\text{g}\cdot\text{ml}^{-1}$ trypsin (Sigma Aldrich)	1 hour at room temperature
<b>blocking buffer</b>	buffer for labelling + 0.4% BSA-c <sup>TM</sup> (Aurion) + 0.1% CWFG gelatin (Sigma-Aldrich)	buffer for labelling + 0.4% BSA	buffer for labelling + 0.4% BSA-c <sup>TM</sup> + 0.1% CWFG gelatin	30 minutes at room temperature
<b>primary antibody</b>	buffer for labelling + anti-sus70 (1 :150) serum + anti-dsDNA (1:200) + 0.4% BSA-c <sup>TM</sup>			overnight at 4°C
<b>secondary antibody</b>	buffer for labelling + anti-IgG-rabbit-gold 15 nm (1:200) + anti-IgG-mouse-gold 5 nm (1:200) + 0.4% BSA-c <sup>TM</sup>			2 hours in an humidity chamber

### **Movie S1. Time-lapse of SPP1 viral-induced compartmentalization of the *B. subtilis* cytoplasm.**

*B. subtilis* GSY10024, producing LacI-mCherry and gp12-mCitrine, was immobilized in a CellAsic microfluidics system and infected with SPP1/*lacO64gp12*<sup>-</sup>. Note that cells are multi-infected. The time-lapse was processed to highlight the phage DNA compartment and virion warehouses as in Fig. 6K (displayed in magenta and green, respectively). Firstly, temporal lateral drift was estimated using the phase contrast channel and corrected on all channels (fluorescence and phase contrast). SPP1 DNA images were pre-processed to remove background signal (rolling ball subtraction, radius = 20 pixels) and enhanced using a Laplacian of Gaussian filter (with an increasing radius from 3 to 5 pixels to respect foci area enlargement during infection). Processed images were segmented using thresholding, converted to binary mask and blurred with a Gaussian filter (radius = 2 pixels) for visualization. Virion warehouses were denoised using median filter (radius: 1 pixel), and enhanced using Laplacian of Gaussian filter (radius = 2 pixels). As soon as warehouses appeared in the time-lapse they were segmented using thresholding, converted to binary mask after rejecting large foci (> 0.5  $\mu\text{m}^2$ ) to remove false detections based on results from Fig. 4C, and blurred with a Gaussian filter (radius = 2 pixels) for the visualization.

### **Dataset S1: Protein and DNA composition of SPP1 viral particles.**

The mass of each protein was calculated from translation of their encoding genes (14). Stoichiometry was derived from structural (32–38) and/or biochemical data (8, 20, 24, 26, 31, 39–43). The mass of DNA encapsidated in SPP1/*lacO64* is identical to SPP1 wild-type (45.1 kbp on average) (44) because SPP1 packages its DNA by a headful packaging mechanism. The genome length of SPP1/*lacO64* used to calculate the mass of non-encapsidated genomes in infected bacteria is 42.3 kbp. An average cell volume of 1.59  $\mu\text{m}^3$  was derived from measurements of *B. subtilis* cells in our experimental conditions. Protein concentrations were then calculated for the total number of subunits engaged in virion assembly. These values aim to provide an order of magnitude of viral polypeptide numbers present in the cell. However, they are not free in the cytoplasm, rapidly assembling into large macromolecular complexes in normal infection conditions. Cumulative numbers for virion components are provided for the SPP1/*lacO64* infection using data from Fig. 1B. Proteins function in viral assembly and their topology in the viral particle are also listed. NA - not applied; NS - non-structural. # - There is an average of 3 gp7 molecules per virion (39). ## - There are 415-420 copies of scaffolding protein gp11 per precursor procapsid (32) but it is not known if the protein is recycled for procapsid assembly after release from procapsids. The mass of gp11 is thus not included in this calculation. ### - The number of subunits of the tape measure gp18 in SPP1 particles is low but not precisely known (37). #### - An encapsidated SPP1 DNA molecule of ~45.1 kbp (44) has a molecular mass of  $\sim 45100 \times 0.65 = \sim 29315$  kDa considering that a base pair has an average molecular mass of 0.65 kDa. A SPP1 genome of 42.3 kbp has a molecular mass of  $\sim 42300 \times 0.65 = \sim 27495$  kDa.

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